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Developing active and intelligent films through the incorporation of grape skin and seed tannin extracts into gelatin

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ABSTRACT

To achieve sustainability in the wine industry, by-products from winery operations are being diverted from waste streams and turned into beneficial use. Grape seed tannin (SeedT) and skin tannin (SkinT) extracts were used to modify the properties of gelatin films, and to prepare active/intelligent films. The SeedT extract showed a higher phenolic content (~ 440 mg gallic acid (GA)/g extract) and antioxidant inhibition (~ 20 %) than the SkinT extract (14 mg GA/g extract, 2 % antioxidant inhibition), while both extracts presented colour variations with an increase of solution pH. The addition of extracts into the gelatin formulation resulted in coloured and transparent films with lower wettability (water contact angle increased up to 92°) and higher UV-light absorbance (secondary antioxidant function) properties. The films were capable of releasing tannins by up to 20 % which led to antioxidant inhibition values of up to 13 % (primary antioxidant function). The addition of SkinT tannins into the films provided the films with a pH indicator ability (intelligent function).

1. Introduction

Nearly all agricultural industries generate wastes and by-products, and winemaking is no exception. To achieve sustainability in the wine industry, by-products from vineyard and winery operations (vine pruning, grape stalk and marc (skins and seeds)) are being diverted from waste and considered for beneficial use (Beres et al., 2017; Maicas & Mateo, 2020). For example, the extraction of chemical compounds from winemaking wastes for use in the health, cosmetics and food industries can be considered a growing area of interest and opportunity for winery by-products valorisation.

In the winemaking process, the grape pomace (also known as grape marc) is the main solid by-product, and it is composed of the remaining skin, seeds and stalks. The grape marc represents nearly 25 % of the total grape weight used in the production of wine. Considering that globally more than 50 million tons of grape crops are produced every year and that approximately 75 % is utilised in winemaking (Beres et al., 2017), the annual grape pomace generation can reach roughly 10 million tons. Seeds and skins are the main components of grape pomace and they represent 38–52 % and 5–10 % of grape marc, respectively. Both are considered sources of structurally diverse phenolic compounds, including larger tannin structures, flavanols and polymeric pigments, which have shown significant antioxidant and antimicrobial activity (Beres et al., 2017; Rockenbach et al., 2011; Silva et al., 2018). Polymeric pigments have also shown pH-sensing properties (Chen, Zhang, Bhandari, & Yang, 2020; Qin, Liu, Zhang, & Liu, 2020; Yong & Liu, 2020). Skin and seed tannins vary in their relative size, amount, subunit composition and sensory properties (Kyraleou et al., 2017).

It is well known that phenolic compounds such as tannins have a significant binding affinity to proteins and that these interactions can result in different structural modifications. These can modify the final properties of protein-based products, such as water repellence (Cano, Andres, Chiralt, & González-Martinez, 2020). The binding is dependent on the type of protein and the diverse structures of the phenolic

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compounds (Jakobek, 2015). On this point, tannins have been reported to have a strong affinity for proline-rich proteins, such as gelatin (Yong & Liu, 2020).

Fish gelatin is an abundant, renewable (extracted from marine wastes), and biodegradable protein, which has shown excellent film-forming ability and transparency, and provides a good barrier to UV-light absorption. These properties have seen gelatin emerge as a green food packaging alternative to petroleum-based single-use food packaging. Gelatin is also an excellent vehicle for incorporating a wide variety of additives and possesses diverse functional groups suitable for physical and chemical modifications (Etxabide, Uranga, et al., 2017). Therefore, the addition of tannin extracts into gelatin formulations can result in gelatin films with enhanced properties while providing films with active and/or intelligent functions.

Active food packaging can release additives previously included in the formulation (e.g. oxygen scavengers, carbon dioxide absorbers/emitters, moisture absorbers, ethylene scavengers, and antioxidant and antimicrobial compounds) into the packaged food or the surrounding environment, to maintain the quality and extend the shelf life of food (Ahmed et al., 2017), while reducing food waste. By contrast, intelligent packaging can be used to monitor O_2 and CO_2, pH, humidity and time-temperature changes (Yousefi et al., 2019), to provide the consumer with more accurate information on the quality and freshness of packaged food. These indicators assist in decision-making processes and can be used to lessen the huge amounts of wholesome edible food that otherwise goes to waste (Ahmed et al., 2017; Yousefi et al., 2019).

The objective of this study is to improve the functionality of gelatin films for active and intelligent packaging applications. For that, grape seed and skin tannin extracts were analysed separately for colour response as well as antioxidant activity. They were then added into gelatin formulations, and the effects of different concentrations on the properties of gelatin films were examined. The colour, UV-Vis light barrier, transparency, water contact angle, interactions between the components, mechanical properties, and morphology of films were assessed. Furthermore, solubility, colour retention, extract release during immersion and antioxidant activity of the released extracts, along with the colour change capacity of the films to external direct and indirect pH stimuli, were examined. To the best of our knowledge, limited studies (Cano et al., 2020) have been reported on the utilisation of tannins for both the modification of proteins such as gelatin films as well as the development of gelatin-based active and/or intelligent packaging systems.

2. Materials and methods

2.1. Materials

An edible (1999/724/CE) codfish gelatin type A with a bloom value of 200 (Weishardt International, Liptovsky Mikulas, Slovakia) was employed as a biopolymer matrix to form films. Edible tannin extracts from grape (*Vitis vinifera* sp) skins (SkinT, in liquid) and seeds (SeedT, in powder) were kindly supplied by Tarac Technologies (Tarac Australia Ltd, Australia). 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin Ciocalteu reagent and gallic acid were purchased from Sigma-Aldrich, while sodium carbonate (Na_2CO_3) and analytical grade ethanol (EtOH) and methanol (MeOH) were obtained from ECP Ltd. (Auckland, New Zealand). 0.4 M NH_4OH, NaOH (0.1–0.4 M) and acetic acid (AAc, 0.1–1.0 M) were prepared and used as pH modifiers.

2.2. Tannin extracts characterisation

2.2.1. Composition analysis of extracts

Monomeric phenolic compounds and tannin subunit composition were determined using an Agilent Technologies (Santa Clara, CA, USA) 1200 series HPLC system that consists of a G1311A quaternary pump, a G1322A degasser, a G2260A autosampler, a G1316A thermostatted column compartment and a G1315D diode array detector. Data were processed with Chemstation for LC 3D systems (Agilent, version B04.02). A reversed-phased C18 column (Kinetex C18, 2.6 μm, 100 Å, 100 mm × 4.6 mm) was used and connected with a Phenomenex C18 guard column (Torrance, CA, USA). Samples (10 g/L in methanol, n = 2) were filtered through 0.45 μm membrane filters (MicroScience, Auckland, New Zealand) into amber glass vials (Thermo Fisher Scientific, Auckland, New Zealand) that were purged with N_2 and immediately analysed. The injection volume was 20 μL, and the column was held at 25 °C. The following detection wavelengths were used for these external standards: 280 nm for gallic acid, (−)-epicatechin, (+)-catechin, vanillic acid, syringic acid; 320 nm for caffeic acid, *p*-coumaric acid, ferulic acid, and *t*-resveratrol; 360 nm for rutin; 520 nm for malvidin-3-glucoside (anthocyanins). Tannin concentration was determined by the methylcellulose tannin precipitation assay [MCP tannins, in (−)-epicatechin equivalents (ECE) g/L]. Spectrophotometric measures were made using a PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). Polymeric pigments, reported in absorbance units (a.u.), were measured with the Harbertson–Adams assay. Spectrophotometric measures were carried out using a PharmaSpec UV-1700 spectrophotometer (Yang, Deed, Araujo, Waterhouse, & Kilmartin, 2022a, 2022b).

2.2.2. Colour response analysis of extracts

Clear colour variations induced by pH modifications were observed with the naked eye for 1 mg/mL SeedT and 5.5 mg/mL SkinT solution (in Milli-Q water (MQW)) and their dilutions (1/2 and 1/5) because a clear colour response to pH variations may be concentration-dependent. It was difficult to observe colour change variations in the solutions with higher tannin-extract concentrations, due to their inherent high colour intensity and darkness. Solutions (100 mL, n = 3) were prepared for each concentration and extract, and then glass transparent bottles were filled with 10 mL of each solution. pH modifiers of different molarities (0.1, 0.2, 0.3, & 0.4 M) of NaOH were used and 40, 20 and 8 μL of a pH modifier was added to the initial, 1/2 and 1/5 solutions, respectively, to examine the effect of external stimuli on colour properties. MQW was added as a control to maintain the extract concentrations. The pH of the solutions was measured using a pH metre and their colour variations and intensity alterations were analysed using a NanoPhotometer (NP80, IMPLEN, Europe) from 235 to 650 nm for each replicate (n = 3).

The colour reversibility of solutions was also analysed by the addition of 40, 20 and 8 μL of 0.4 M of NaOH and AAc solutions, in turn, two times, into the tannin extract solutions.

2.2.3. Total phenolic content (TPC) and antioxidant activity of extracts (DPPH assay)

TPC values were determined using the Folin-Ciocalteu assay (Etxabide, Coma, et al., 2017) on 1 mg/mL extract concentration. All tests were carried out in triplicate and TPC was expressed as mg gallic acid (GA)/g extract (GA, calibration curve (0–1 mg/mL): y = 0.0104 + 0.6308x, $R^2 = 0.998$).

DPPH radical scavenging activity was measured according to the method of Etxabide, Uranga, et al. (2017) with slight modification. 55 μg/mL of tannin solutions were prepared in triplicate (n = 3) and 0.1 mL of a sample was mixed with 3.9 mL of DPPH solution (75 μM) in MeOH. The mixtures were vigorously shaken and allowed to stand at room temperature in the dark for 30 min. Free radical scavenging activity was expressed as mg GA/g extract (GAE, calibration curve (2.5–100 μg/mL): y = 0.01904 + 0.73455x, $R^2 = 0.999$). The inhibition values (*I*) were determined by the absorbance decrease at 518 nm as follows:

$$I \quad (\%) = \quad \frac{A_c - A_s}{A_c} \quad \times 100 \qquad (1)$$

where A_c is the absorbance of the MQW and A_s is the absorbance of the tannin extract solutions.

2.3. Gelatin film characterisation

2.3.1. Film preparation

Fish gelatin-based films with different tannin extract contents were prepared by casting. Before proceeding with making tannin extract-containing films, checks were made so that the temperature used in the film preparation method did not compromise the stability of tannin extracts (Fig. S1). One gram of gelatin was dissolved in MQW (20 mL) for 30 min at 60 °C and 250 rpm to obtain a homogeneous blend of the film-forming solution (FFS). After that, tannin extracts (SeeT: 0.5 %, 1 %, 2 %, 3 %, & 4 % (higher amounts did not end up dissolving properly); SkinT: 2.75 %, 5.5 %, 11 %, 16.5 %, & 22 % (higher amounts resulted in highly coloured films) (% on a gelatin dry weight basis)) were added into the FFS which was then mixed for 30 min at 60 °C and 1000 rpm. Finally, 6.00 ± 0.1 g of FFS was poured into each Petri dish (ø 90 mm) and left to dry and form the film in an air-circulating fume hood at 20 °C and 56 % RH for 24 h. The samples were then peeled from the dishes and named as SeedTx and SkinTx, with the "x" value representing the added tannin extract concentration. Films without extracts were prepared as control samples. All films were stored at 20 °C and 63 % RH for 48 h before testing.

2.3.2. Colour, transparency and light barrier properties of films

The colour of the films was determined with a colourimeter CR-300 Minolta (Konica Minolta, Japan), previously calibrated on a white standard calibration plate. Films were placed onto the surface of a white paper sheet (L* = 91.37, a* = 7.07 and b* = − 11.24) and then L* , a* and b* colour parameters were measured using the CIELAB colour scale: L* = 0 (black) to L* = 100 (white), − a* (greenness) to + a* (redness), and − b* (blueness) to + b* (yellowness) (n = 9). Total colour difference (ΔE*) values for films with tannin extracts, as a function of their concentration, were calculated in reference to the control films (placed onto a white paper sheet) as follows (Etxabide, Maté, et al., 2021):

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

The NanoPhotometer was used to assess the effect of the addition of different tannin extract concentrations on the barrier properties of the films to ultraviolet (UV) and visible (vis) light at wavelengths from 235 to 650 nm. The transparency (light transmittance) of the films was determined at 600 nm as follows:

$$T = \frac{A_{600}}{x} \quad (3)$$

where A_{600} is the absorbance value at 600 nm and x is the average thickness (mm) of the films. Higher T values indicate a lower light transmittance value (Garrido, Etxabide, Guerrero, & de la Caba, 2016).

2.3.3. Water contact angle (WCA) determination

WCA measurements were performed by placing a 4 μL droplet of MQW on different sample surface regions to estimate the wettability of the gelatin films. An optical contact angle measuring instrument (CAM 100, KSC instruments Ltd., Finland) was used. The image of the drop was taken using CAM100 software and ten measurements (n = 10) were taken for each composition at 20 °C and 67 % RH (Etxabide, Kilmartin, et al., 2021).

2.3.4. Attenuated Total Reflection-Fourier Transform Infra-red (ATR-FTIR) spectroscopy

FTIR spectra of the individual components, as well as the films, were recorded using a single bounce Platinum Diamond Micro-ATR accessory on a Bruker Vertex 70 FTIR spectrometer (Bruker Optics, New Zealand). A total of 32 scans at 4 cm^{-1} resolution were performed and the measurements were recorded between 4000 and 800 cm^{-1}. The OPUS 7.5 software was used to undertake the normalisation of each spectrum (Etxabide, Kilmartin, et al., 2021).

2.3.5. X-ray diffraction (XRD)

XRD studies of films fixed on the top of a sample holder were performed with a diffraction unit (Empyrean, Malvern Panalytical, Cleveland, New Zealand) operating at 45 kV and 40 mA. The radiation was generated from a Cu-Kα (λ = 1.5418 Å) source and the diffraction data were collected from 2θ values from 3° to 50°, where θ is the incidence angle of the X-ray beam on the sample (Etxabide, Kilmartin, et al., 2021).

2.3.6. Mechanical properties

Young's modulus (E), ultimate tensile strength (UTS) and elongation at break (EB) were measured for each film at least six times on an Instron 5943 mechanical testing system (Instron, Norwood, USA) according to ASTM D882-02 (2002). Sample preparation was carried out by cutting the films into strips of 50 mm × 10 mm, and the test was run at a gauge length of 30 mm and 1 mm/min crosshead speed with a 50 N load cell (Etxabide et al., 2021).

2.3.7. Scanning electron microscopy (SEM)

Samples were torn apart and fixed on glass slides using carbon tapes and sputter-coated with gold for 2 min using a sputter coater (Quorum Q150R S, Australia). SEM (JCM-6000 Versatile Benchtop SEM, Korea) was used to observe the cross-section morphology of films at an accelerating voltage of 15 kV.

2.3.8. Solubility of films

Three specimens (n = 3) of each film were weighed (W_0) and immersed in 4.5 mL of MQW and 50 % (v/v) ethanol (50EtOH) food simulants (Commission Regulation EU No 10/2011, 2011) for 24 h at different storage temperatures to simulate chilled (4 °C) and ambient (22 °C) commercial food storage conditions. After that, specimens were taken out of the food simulants and left to dry in an air-circulating fume hood at room temperature (20 °C) for 24 h before reweighing (W_t). The solubility (S) of films was calculated using the following equation:

$$S \quad (\%) = \frac{(W_0 - W_t)}{W_0} \times 100 \quad (4)$$

2.3.9. Tannin extracts release from films and their antioxidant activity

A NanoPhotometer was used to determine the cumulative release of extracts into 50EtOH food simulant under chilled storage conditions. First, the wavelength of maximum absorbance for extracts was measured (λ_{max} = 280 nm) and the standard solutions were prepared to establish the calibration curves for SeedT (y = 0.029 + 17.070x, R^2 = 0.999 (0.5–0.062 mg/mL)) and SkinT (y = − 0.033 +1.827x, R^2 = 0.999 (1–0.25 mg/mL)). Films (2 cm × 4 cm) were immersed in 4.5 mL of 50EtOH and stored in a fridge at 4 °C for 24 h. At particular time intervals (0.5, 1, 2, 4, 8, & 24 h) aliquots of 1.5 mL were withdrawn from the simulant and replaced with an equal volume of fresh 50EtOH to maintain the initial immersion volume. The aliquots were analysed by spectroscopy at 280 nm and control films were used as a reference. All tests were carried out in triplicate (n = 3) and the results are expressed as a percentage of tannin cumulative release with respect to the tannin incorporated in the films.

The DPPH assay was carried out in 50EtOH, in which films (2 cm × 4 cm) were immersed in 50EtOH (4.5 mL) at 4 °C for 24 h. The inhibition percentage was measured as described in Section 2.2.2, with A_c being the absorbance of the simulant in which the control samples were immersed, and A_s the absorbance of the simulant where extract-containing samples were immersed.

2.3.10. Colour retention of the films

The capacity of films to retain the colour (L*, a*, and b*) after immersion in 50EtOH at 4 °C for 24 h was measured using a colorimeter.

Total colour difference (ΔE*) values for dried films were calculated as described in Section 2.3.2, but films with the same composition before immersion were taken as references. ΔE* was used to analyse whether there were visually predictable changes in the colour of films before and after immersion.

2.3.11. Colour response analysis of films

Films (2 cm × 2 cm) were deposited onto square (2 cm × 2 cm) paper sheets previously damped with 40 μL of 0.1 M NaOH to measure the colour response of films containing different types and concentrations of tannins to a basic pH surface. The films stuck onto the damped paper sheets were dried in an air-circulating fume hood at 20 °C and 56 % RH for 4 h and the total colour difference (ΔE*) values for films, as a function of tannin type and concentration, were calculated and referred to the same composition of films damped with MQW. This test was considered as a direct (films in contact with the damped surface) pH modification of the films.

For the indirect (films in contact with the vapour) pH modification test, films were placed in the headspace (inner phase of the lid) of sealed containers (18 cm × 12 cm × 4 cm) loaded with 15 mL of 0.4 M NH_4OH in the bottom of the container (Yun et al., 2019). Every 10 min for 30 min, films were taken out and placed onto the surface of a white paper sheet to measure the colour changes using the colourimeter. ΔE* values were calculated and referenced to the films not exposed to NH_4OH. Three specimens (n = 3) were tested for each composition and time of exposure.

2.4. Statistical analysis

Data were subjected to a one-way analysis of variants (ANOVA) through an SPSS computer program (SPSS Statistic 25.0). Post hoc multiple comparisons were determined by Tukey's test with the level of significance set at $p < 0.05$.

3. Results and discussion

3.1. Tannin extract characterisation

Tannins were analysed using HPLC, methylcellulose tannin precipitation, and Harbertson–Adams assays (Table S1). The SkinT product contained around 92 % of water, and fewer tannins (~ 1 %) than SeedT (~ 63 %). SeedT contained a lower yield than SkinT which indicated more tannin macromolecules in SeedT that could not be easily depolymerised. The SkinT extract presented higher degrees of polymerisation (mDP), trihydroxylation (i.e., (-)-epigallocatechin extension units) and galloylation (i.e., (-)-epicatechin-3-*O*-gallate extension and terminal

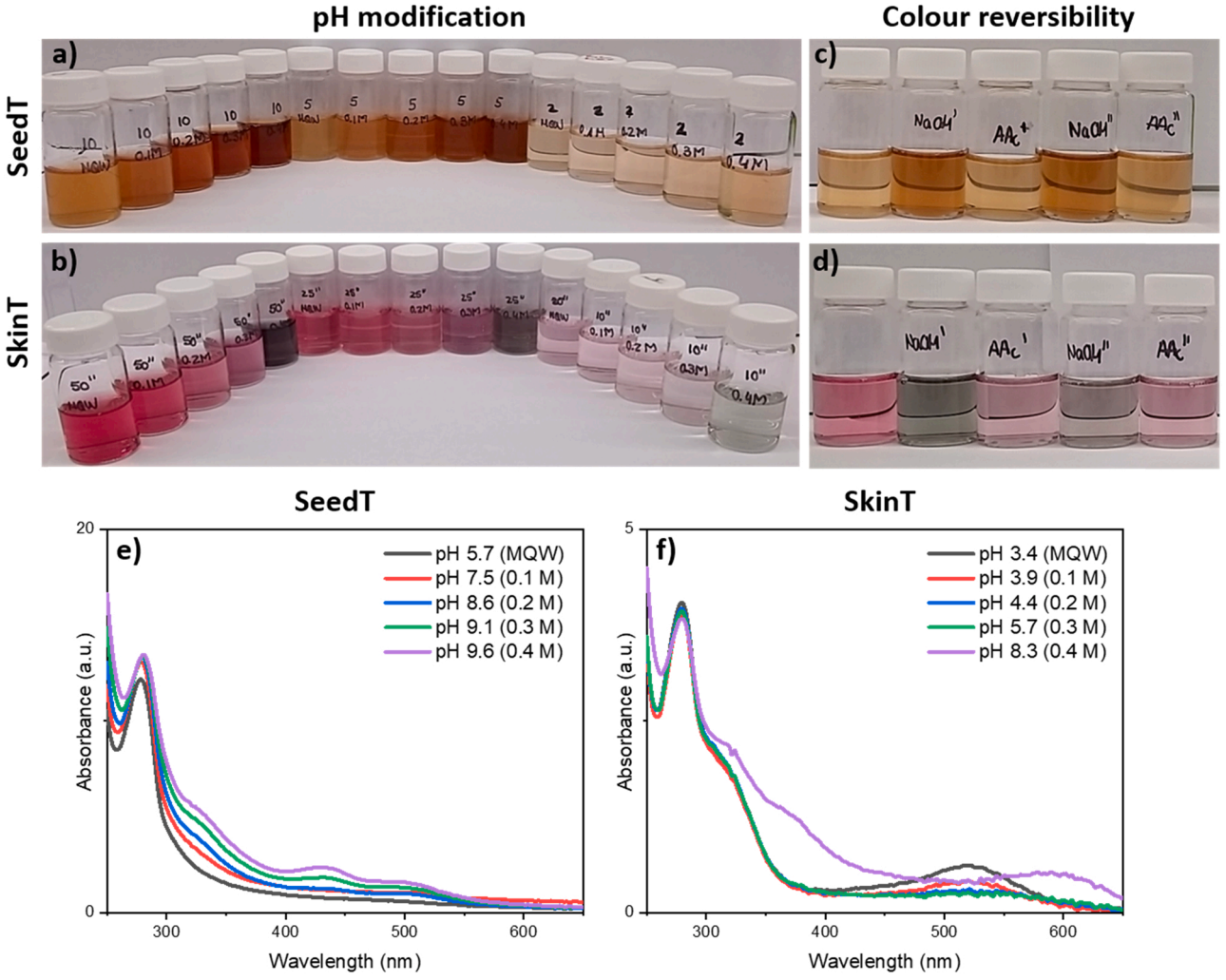

Fig. 1. The colour variation of a) seed tannin (SeedT) and b) skin tannin (SkinT) extracts in solution as a function of concentration (in blocks of five samples: initial concentration and their dilutions (1/2 and 1/5) from left to right), and pH (for each block of five: Milli-Q water (MQW), and 0.1, 0.2, 0.3, 0.4 M NaOH, from left to right); colour reversibility of c) SeedT and d) SkinT extract 1/2 diluted solution with the addition of 0.4 M of both NaOH and acetic acid (AAc) solutions in turns two times; UV-Vis spectra of e) SeedT and f) SkinT extract initial concentrations as a function of pH.

units), which was more than 70 % of the total tannin subunits released after depolymerisation. Regarding SeedT, (-)-epigallocatechin extension units were also detected indicating the influence from grape skins during commercial extraction. Unlike SeedT, SkinT contained polymeric pigments of red colour. Both products contained very minimal amounts of monomeric phenolics, where gallic acid, (+)-catechin, and (-)-epicatechin were detected in the seed tannins while skin tannins only had (+)-catechin. Other compounds such as monomeric anthocyanins, vanillic acid, syringic acid, caffeic acid, ferulic acid, among others, were not found (< D.L.) in the extracts.

3.1.1. Effect of concentration and pH on colour change in extracts

The suitability of tannin extracts as pH indicators was first examined as a function of tannin extract concentration, since a clear colour response to pH variations may be concentration-dependent (Etxabide, Kilmartin, et al., 2021; Pourjavaher, Almasi, Meshkini, Pirsa, & Parandi, 2017). Initial solutions of SeedT and SkinT extracts (Fig. 1 **a** and **b**,) were yellow-brownish and ruby-reddish, respectively, and their colour intensity decreased as the concentration of the extract decreased. These colour differences between extracts can be related to the content of structurally diverse phenolics as well as the type and concentration of polymeric pigments in the extracts (Table S1), which are responsible for the colour of red wines (Zhang et al., 2017). Contrary to the decrease in pH, alkalinisation of the extract solutions led to colour and intensity changes (Fig. 1 **a** and **b**,). The pH increase from pH ≈ 5–6 (MWQ) to pH ≈ 9–10 (with the addition of 8–40 μL drops of 0.4 M NaOH) in SeedT showed more intense yellow-brownish colours, which became darker as the solutions became more alkaline. These changes were also observed as an increase in light absorbance (Fig. 1 **e**) in the visible range (370–650 nm) and especially for bands centred at 430 nm and 510 nm (perceived complementary colours: yellow and purple, respectively), as the pH increased. The mix of colours resulted in yellow-brownish solutions with higher intensities as the pH increased (Fig. 1 **a** and **e)**. In the literature, these intensity and colour variations were related to non-enzymatic (chemical) oxidation processes (Guo, Yang, Tang, & Yuan, 2020). The browning processes can modify the structure of the extracted phenolic grape tannins and they are accelerated at a higher pH.

In the SkinT solutions, however, the increase in pH induced colour and intensity changes (Fig. 1 **b** and **f**): the addition of some drops (8–40 μL) of 0.1–0.3 M NaOH (pH of the extract containing solutions ≈ 4–6) resulted in a decrease of the band centred at 520 nm (perceived complementary colour: purple), which led to less coloured solutions. However, the addition of some drops of 0.4 M NaOH (pH ≈ 8) induced a notable absorbance variation, since a more intense shoulder, centred at 380 nm (complementary colour: yellow) and a band centred at 600 nm (complementary colour: green-blue) appeared, which gave the characteristic colour to these samples (Fig. 1 **b**). These colour variations can be related to molecular structural transformations of polymeric pigments (Table S1) (Chen et al., 2020; Kennedy & Waterhouse, 2000; Zumdahl & DeCoste, 2013). However, the decrease in extract concentration (dilution 1/2 and 1/5) did not result in new colour variability, contrary to previous studies (Etxabide, Kilmartin, et al., 2021; Pourjavaher et al., 2017), which could be related to the dilution ratio. Both extracts showed a reversible colour change in alkaline environments (Fig. 1 **c** and **d**) and so, are suitable as pH indicators for foodstuffs that release chemicals that alkalify the food and the packaging headspace, such as meat (e.g., beef) and seafood (e.g., shrimps) that release volatile amines during spoilage (Sobhan, Muthukumarappan, & Wei, 2022).

It is worth mentioning that both extract solutions had a strong absorption band at 280 nm and an inflexion point minimum at 258 nm (Fig. 1 **e** and **f**), which indicated that the main components in the extracts were condensed tannins (Falcão & Araújo, 2013). However, the absorbance increase at 280 nm could also be related, to a lesser extent, to the presence of monomeric phenolic compounds in the extracts (Table S1) (Arrieta, Sessini, & Peponi, 2017; Stevenson et al., 2019). Furthermore, as observed in Table S1, the tannin content was significantly higher in the SeedT extract, since lower concentrations were used (1 mg/mL and its dilutions), and higher absorbance values were obtained with the SeedT extract compared to the SkinT extract (5.5 mg/mL and its dilutions).

3.1.2. Total phenolic content and antioxidant activity of the extracts

Phenolic compounds such as tannins are known to be natural antioxidant compounds and so the suitability of tannin extracts as active components was examined (Table S2). As expected, SeedT extract presented TPC and inhibition values about 30 and 10 times higher, respectively, than SkinT extract. This was related to more phenolic compounds in the latter extract, as seen in Fig. 1 **e** and **f,** and Table S1. The antioxidant properties of the extracts, especially SeedT, showed that these compounds could be used for antioxidant (active) film development (Cano et al., 2020; Riahi, Priyadarshi, Rhim, & Bagheri, 2021).

3.2. Gelatin films characterisation

3.2.1. Optical properties of the films

Colour and transparency are relevant characteristics of packaging, since they directly influence product appearance and visibility, and thus, consumer acceptability (Monedero, Fabra, Talens, & Chiralt, 2009). The presence of the extracts in the films caused a decrease in L* values (lightness reduction), especially in the SkinT films (Table 1), which was linked to a reduction in transparency due to the extract's visible light absorbance capacity (Fig. 1 **e** and **f**) (Mir, Dar, Wani, & Shah, 2018). However, the films remained transparent (Fig. S2), even at the highest extract concentrations, and they presented lower T values (higher transparency) than commercial films, such as LDPE films (T value of 4.26, (Guerrero, Stefani, Ruseckaite, & de la Caba, 2011)). Unlike the L* values, the a* values increased (toward redness) as the extract concentration increased, irrespective of extract type. As for b*, the values notably increased (toward yellowness) with the addition of SeedT extract, due to its yellow-brownish colour, while no significant variations were observed when SkinT was added, irrespective of its concentration. Therefore, the total colour difference (ΔE*) (Table 1) increased and the effects were greater when higher concentrations of extract were used, irrespective of extract type. It is worth mentioning that the SeedT extract had a higher capacity to colour films since much lower concentrations were required to obtain the same ΔE* values as with SkinT extracts.

Considering that the intelligent film is generally one part (e.g., indicator label placed on or within the packaging material) of the whole packaging, it is of utmost importance that the addition of tannins induces a visible colour change. Thus, further colour changes induced by pH may also be visually detected. SeedT percentages of 1 and 2 (SeedT1 & SeedT2) and SkinT percentages of 11 and 16.5 (SkinT11 & SkinT16.5), were the minimum amounts of extract required to induce perceptible colour differences ($3.0 < \Delta E < 6.0$ (Paciulli, Palermo, Chiavaro, & Pellegrini, 2017)) within the gelatin films (Table 1). Statistically, SeedT1 & SkinT11, as well as SeedT2 & SkinT16.5 presented the same ΔE* values. Therefore, these 4 formulations plus control films, were selected for additional characterisations of the developed films. Based on the selected films' tannin extract concentrations as well as the tannin percentage in each extract (Table S1), the tannin content in each film was estimated: ~ 1.88 mg (SeedT 1); ~ 3.76 mg (SeeT 2); ~ 0.40 mg (SkinT11); and ~ 0.60 mg (SkinT16.5).

3.2.2. Light barrier properties and wettability of films

Considering that light can lead to vitamin degradation, discolouration of fresh food, development of off-flavours and auto-oxidation of fats (Duncan & Chang, 2012), protection against the light (UV and visible light) is a critical role in food packaging. Therefore, the UV-Vis light absorption capacity of the gelatin films was analysed as a function of

Table 1
Thickness, transparency (T) and colour parameter (L*, a*, b*, ΔE*) values of gelatin films as a function of seed tannin (SeedT) concentrations (0.5–4 %), and skin tannin (SkinT) concentrations (2.75–22 %) (on a gelatin dry weight basis). Films without tannins were used as control samples. Two means followed by the same letter in the same column are not significantly different ($p > 0.05$) using Tukey's multiple range test.

Films	Thickness (μm)	T	L*	a*	b*	ΔE*
Control	31.2 ± 2.3^{a}	1.30 ± 0.11^{a}	90.84 ± 0.02^{a}	6.90 ± 0.03^{a}	-10.62 ± 0.02^{a}	–
SeedT 0.5	32.1 ± 1.3^{a}	1.55 ± 0.35^{ab}	90.09 ± 0.10^{abc}	7.04 ± 0.01^{ab}	-8.91 ± 0.10^{b}	1.87 ± 0.12^{b}
SeeT 1	34.8 ± 6.4^{a}	1.81 ± 0.34^{ab}	89.46 ± 0.10^{bc}	7.25 ± 0.04^{c}	-7.28 ± 0.27^{c}	3.62 ± 0.28^{c}
SeedT 2	33.2 ± 6.2^{a}	1.91 ± 0.43^{ab}	88.51 ± 0.24^{de}	7.56 ± 0.08^{d}	-5.11 ± 0.69^{d}	6.01 ± 0.73^{d}
SeedT 3	33.8 ± 0.3^{a}	1.84 ± 0.02^{ab}	87.40 ± 0.28^{f}	8.08 ± 0.04^{e}	-1.89 ± 0.11^{e}	9.45 ± 0.12^{f}
SeedT 4	35.3 ± 5.0^{a}	2.01 ± 0.20^{ab}	86.12 ± 0.08^{g}	8.47 ± 0.12^{f}	-0.51 ± 0.02^{f}	11.27 ± 0.03^{g}
SkinT 2.75	34.4 ± 5.2^{a}	1.32 ± 0.20^{a}	90.31 ± 0.06^{ab}	7.11 ± 0.03^{bc}	-10.52 ± 0.10^{a}	0.58 ± 0.06^{a}
SkinT5.5	33.2 ± 6.7^{a}	1.45 ± 0.25^{ab}	89.36 ± 0.22^{cd}	7.61 ± 0.02^{d}	-10.36 ± 0.09^{a}	1.66 ± 0.22^{b}
SkinT 11	29.9 ± 5.4^{a}	1.87 ± 0.33^{ab}	87.69 ± 0.30^{ef}	8.47 ± 0.13^{f}	-10.34 ± 0.04^{a}	3.52 ± 0.32^{c}
SkinT 16.5	30.9 ± 5.4^{a}	2.16 ± 0.27^{b}	86.15 ± 0.19^{g}	9.35 ± 0.08^{g}	-10.48 ± 0.07^{a}	5.29 ± 0.21^{d}
SkinT22	35.6 ± 5.3^{a}	2.15 ± 0.27^{b}	84.37 ± 0.81^{h}	10.22 ± 0.07^{h}	-10.65 ± 0.13^{a}	7.28 ± 0.76^{e}

extract type and concentration to study the effect of tannin extract addition on the light barrier properties of gelatin films (Fig. 2 **a** and **b**). A notable increase in UV-light absorbance at 280 nm was observed, which indicated the presence of tannins in the films (Grasel, Ferrão, & Wolf, 2016). The improvement of the UV absorbing property of the films can be considered a preventive (secondary) antioxidant function in active packaging, since this enhancement may provide films with the capacity to lower the occurrence of photo-oxidation reactions in foods (Vilela et al., 2018). It is worth mentioning that these values were much better than those of synthetic films used on the market, which showed no barrier property to UV light (Guerrero et al., 2011). Unlike SeedT films, SkinT films also showed an increase in a band centred around 520 nm, related to polymeric pigments, which provided films with a ruby-reddish colour (Table S1). It is worth mentioning that although higher concentrations of SkinT extract were used, SeedT films showed slightly higher light absorbance values, and so better protection against light, especially in the UV range (~ 200–400 nm). No major improvements were seen in the visible region (~ 400–650 nm), regardless of the tannin extract type. Taking into account that the UV region of light is considered very harmful to food quality (Duncan & Chang, 2012), these films could be suitable to protect and extend food quality.

Surface characteristics such as the wettability of films are important properties, since, depending on the hydrophilicity/hydrophobicity of the film surface, interactions among the components in food and films can vary. This could in turn affect the properties of films, such as the pH-sensing ability or the release of phenolic compounds. Therefore, the wettability of the films as a function of tannin extract type and concentrations was studied (Table 2). The addition of tannin extract notably lowered the hydrophilicity of the films, irrespective of extract type. However, unlike SkinT extracts, an increase in SeedT concentration resulted in hydrophobic films (WCA > 90°), (Sethi & Manik, 2018). This wettability reduction in films could be related to the presence of molecular interactions such as hydrogen bonds between hydroxyl groups of the tannins and polar groups of gelatin, as well as hydrophobic interactions (Yong & Liu, 2020). It seemed that the addition of SeedT extract formed more interactions with gelatin than the SkinT extract since greater WCA values were observed in these films. This could be related to a higher presence of phenolic compounds in SeedT extract compared to the SkinT extracts (Tables S1 and S2), which could promote cross-linking between the gelatin chains through hydrophobic

Table 2
Water contact angle (WCA), Young's modulus (E), ultimate tensile strength (UTS), and elongation at break (EB) values for gelatin films with 1 % and 2 % seed tannin extract (SeeT1 & SeedT2), and 11 % and 16.5 % skin tannin extract (SkinT11 & SkinT16.5) (on a gelatin dry weight basis). Films without tannins were used as control samples. Two means followed by the same letter are not significantly different ($p > 0.05$) through Tukey's multiple range test.

	WCA (°)	E (MPa)	UTS (MPa)	EB (%)
Control	79.8 ± 2.7^{a}	2717.7 ± 163.8^{a}	65.2 ± 6.0^{a}	4.5 ± 0.2^{ab}
SeedT1	85.4 ± 1.7^{b}	2805.4 ± 151.7^{a}	65.8 ± 2.2^{a}	4.3 ± 0.3^{ab}
SeedT2	92.4 ± 2.5^{c}	2862.0 ± 97.8^{a}	68.0 ± 6.1^{a}	4.5 ± 0.3^{ab}
SkinT11	86.8 ± 1.9^{b}	2650.9 ± 276.5^{a}	68.5 ± 5.0^{a}	4.2 ± 0.6^{a}
SkinT16.5	86.2 ± 1.2^{b}	2746.3 ± 177.0^{a}	67.0 ± 8.0^{a}	5.0 ± 0.4^{b}

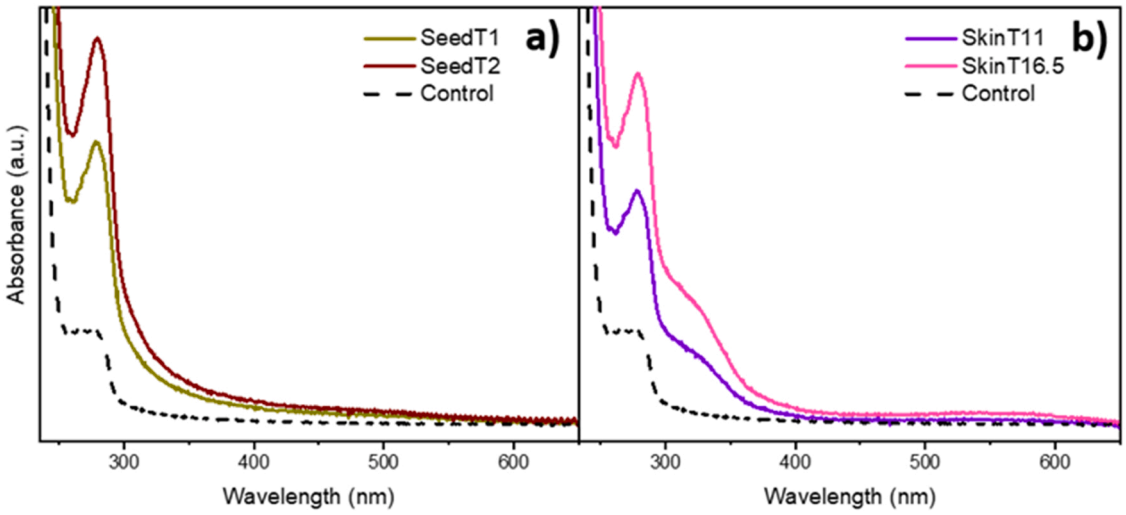

Fig. 2. UV-Vis spectra for gelatin films with a) 1 % and 2 % seed tannin extract (SeeT1 & SeedT2), and b) 11 % and 16.5 % skin tannin extract (SkinT11 & SkinT16.5) (on a gelatin dry weight basis). Films without tannins were used as control samples.

interactions and may have led to increased water barrier properties as seen by Mushtaq and co-workers on protein-based films containing pomegranate peel extract (Mushtaq, Gani, Gani, Punoo, & Masoodi, 2018).

3.2.3. *Physicochemical, structural, mechanical, and morphological properties of films*

To better understand the chemical affinity between the compounds, protein-extract interactions were studied by FTIR analysis, and the spectra are shown in Fig. 3. The spectra of tannin extracts (Fig. 3 **a** and **b**) showed a broad band centred at 3268 cm^{-1} and a small band at 2932 cm^{-1} which are related to O-H and C-H stretching, respectively. The bands at 1723, 1604 and 1516, 1444 cm^{-1} are associated with C═O stretching, COO^-, and C-C aromatic stretching vibrations, respectively. The bands in the range 1140–900 cm^{-1} are ascribed to the C-O and C-C vibrations of polysaccharides (Kyraleou et al., 2017; Nogales-Bueno et al., 2017). Some variations between SkinT and SeedT spectra were observed, probably due to the structural diversity of the components in the extracts (Table S1) (Falcão & Araújo, 2013; Watrelot & Norton, 2020).

The addition of the extracts into the gelatin films (Fig. 3 **c** and **d**) caused no significant changes, compared to control spectra, since the main absorption bands of gelatin, related to N-H and O-H stretching at 3295 cm^{-1} (Amide A), C═O stretching at 1630 cm^{-1} (amide I), N-H bending at 1545 cm^{-1} (amide II) and C-N stretching at 1239 cm^{-1} (amide III) (Frazier & Srubar, 2016), were similar. However, the band associated with amide A shifted to higher wavenumbers (from 3295 cm^{-1} in the control to 3301 cm^{-1} in SeedT2) and became wider and sharper when the extracts were incorporated. These changes were more notable as the extract concentration increased, especially with SeedT additions. Similar results were found in the literature where the band associated with the stretching vibration of N-H bands between 3250 cm^{-1} to 3600 cm^{-1} became wider and sharper when antioxidant extracts such as green tea extract, grape seed extract, ginger extract, and ginkgo leaf extract were incorporated into gelatin films (Li, Miao, Wu, Chen, & Zhang, 2014). These changes were related to the presence of hydrogen bonds between the functional groups of extracts and gelatin.

Regarding the tertiary structure of gelatin, the XRD results of all gelatin films (Fig. 4 **a and b**) displayed two diffraction peaks: a sharp peak (7.5°), corresponding to the residual triple-helical structure of native collagen, and a broad peak (20°), associated with the partial crystalline structure of gelatin (Liu, Antoniou, Li, Ma, & Zhong, 2015). The addition of extracts did not affect the rearrangement of the triple-helix and the reordering of the gelatin crystalline region, irrespective of extract type and concentration, since no significant changes in peak intensities were observed. This can be ascribed to the low percentages of SeedT extracts used (1 % and 2 %), as well as the low amount of phenolic compounds in the SkinT extracts (Table S1).

Taking into account that the triple-helix content of gelatin can be related to the mechanical properties of gelatin-based materials (Bigi, Panzavolta, & Rubini, 2004), films were analysed to determine their mechanical characteristics (Table 2). As expected, the addition of extracts did not produce significant changes in the mechanical properties of the films, since both the triple-helix content and the crystallinity of the gelatin films did not significantly vary with the incorporation of tannins. This is seen in Young's modulus, ultimate tensile strength (UTS) and elongation at break (EB) values (Table 2), which did not show considerable variations when different amounts of the extracts were added. These results indicated that the addition of extracts did not induce the development of heterogeneous structures with the presence

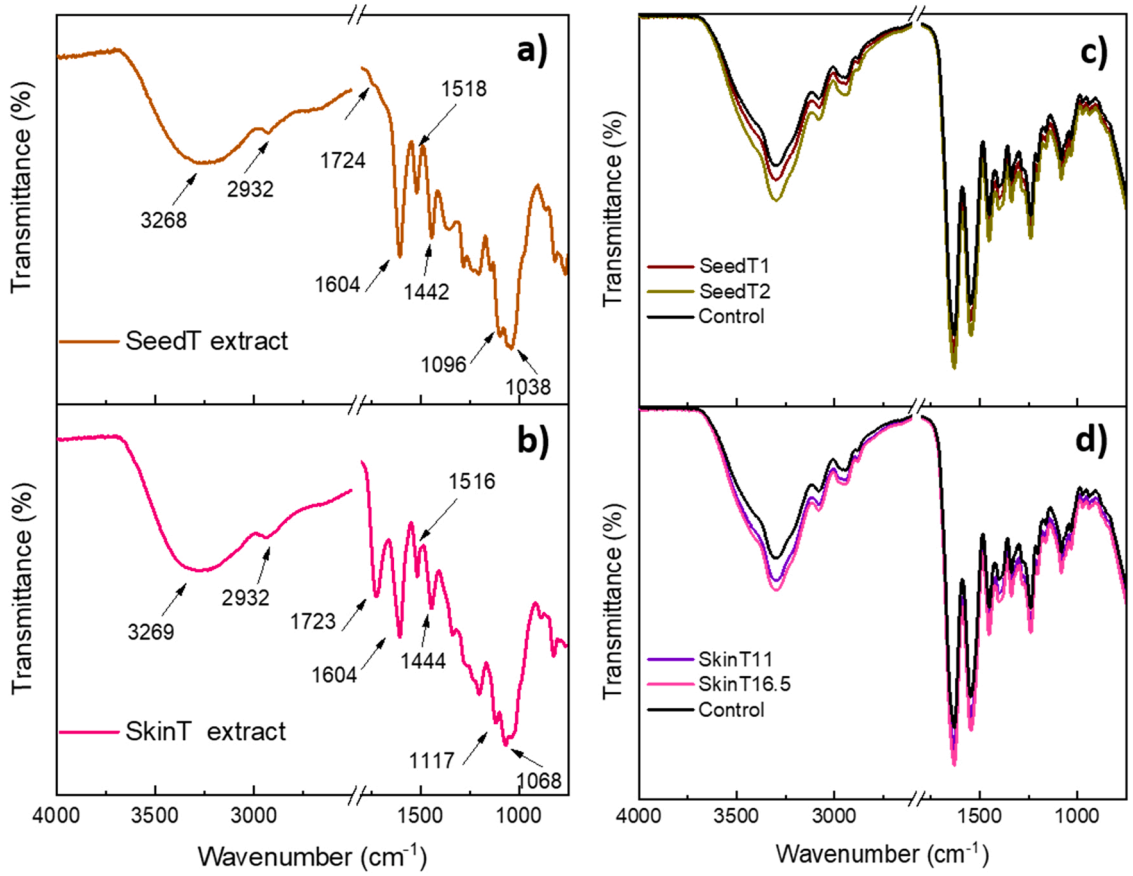

Fig. 3. FTIR spectra of a) seed tannin (SeedT) and skin tannin (SkinT) extracts and gelatin films with c) 1 % and 2 % seed tannin extract (SeeT1 & SeedT2), and d) 11 % and 16.5 % skin tannin extract (SkinT11 & SkinT16.5) (on a gelatin dry weight basis). Films without tannins were used as control samples.

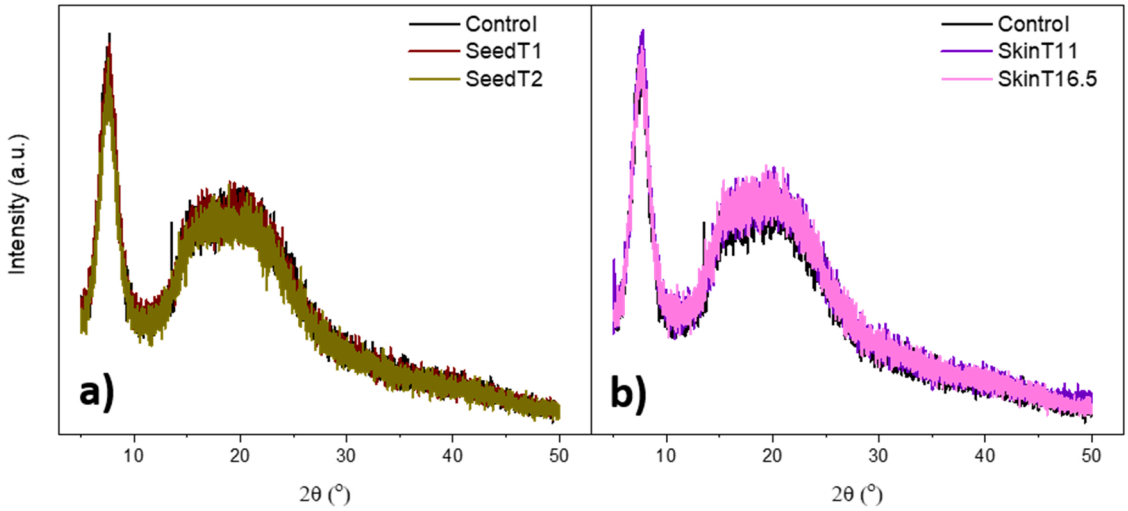

Fig. 4. XRD patterns of the gelatin films as a function of added extract type and their concentration: a) 1 % and 2 % seed tannin extract (SeeT1 & SeedT2), and b) 11 % and 16.5 % skin tannin extract (SkinT11 & SkinT16.5) (on a gelatin dry weight basis). Films without tannins were used as control samples.

of discontinuous areas, which could have compromised the mechanical properties of films, as seen by Li et al. (2014) in active gelatin-based films incorporated with natural antioxidants. This can be demonstrated by the SEM results (Fig. 5) where images of the cross-section of films showed continuous and uniform films even after the addition of extracts (differences in roughness were probably related to the film fracture process rather than the film morphology itself). This result indicated that the incorporation of tannins did not cause changes in both the tertiary and inner structure of the films, and that gelatin and the tannin extracts were homogeneously mixed (Liu et al., 2015).

3.2.4. Solubility, release of extracts, antioxidant activity, and colour retention of films

The state, composition, and storage of food (e.g. solid, liquid; fat/water content; chilled, ambient temperature, etc.) determines the type of material to be selected for food packaging. Therefore, in this study, different food simulants and storage temperatures were used to analyse the solubility of gelatin-based films with different tannin extracts. The solubility of the films (Table 3) was reduced with a decrease in both storage temperature and water content of the food simulant, irrespective of film formulation, due to the relatively low melting point (around 30 °C) and the hydrophilic nature of gelatin (Stevenson et al., 2019). The outcomes showed that solubility decreased as follows: MQW-22 °C (100 %) > 50EtOH-22 °C (~ 30 %) > MQW-4 °C (~ 2 %) ≈ 50EtOH-4 °C (~ 2 %), irrespective of extract type and concentration. The results indicated that films were suitable for lipophilic foods and more adequate for chilled food. However, unlike 50EtOH-4 °C storage conditions, films stored in MQW-4 °C and 50EtOH-22 °C presented changes in their size (swelled/-shrunk, respectively, Fig. S3) after immersion. Physical changes in films may affect the properties of films such as colour intensity (Etxabide,

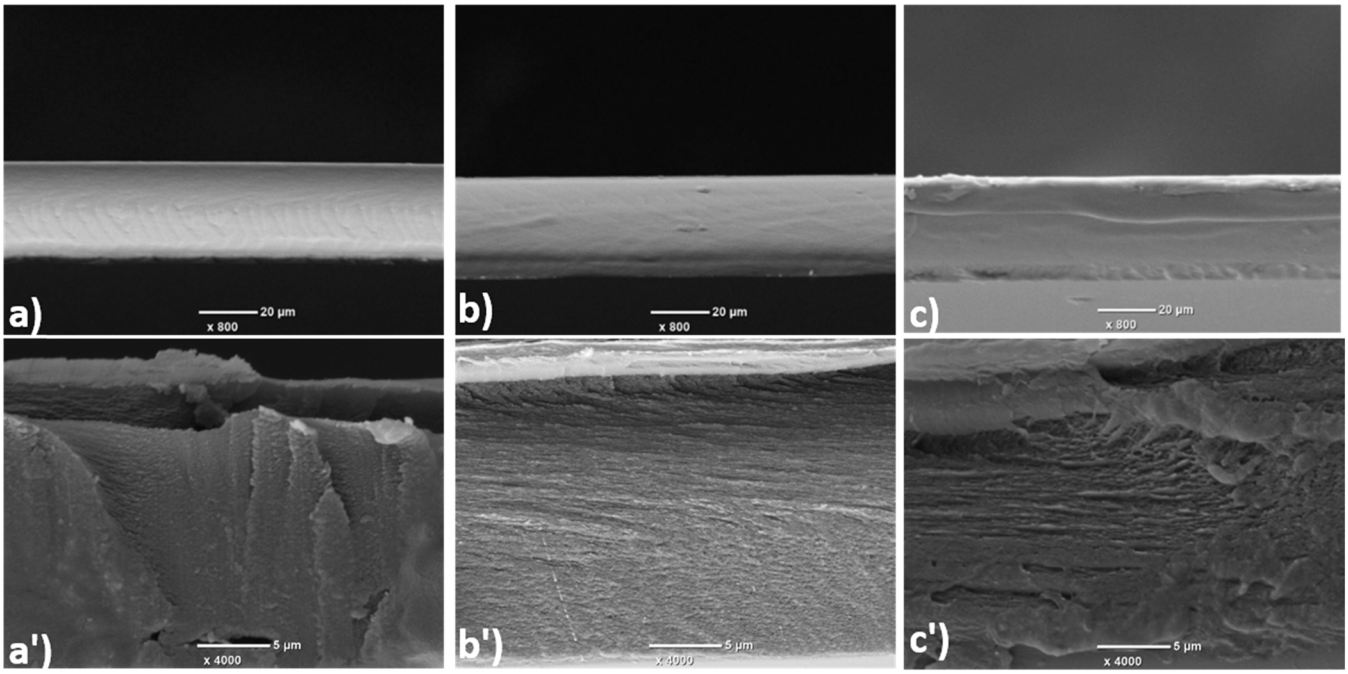

Fig. 5. SEM cross-sectional micrographs of films a–a′) control, with b–b′) 2 % seed tannin extract (SeeT2) and c–c′) 16.5 % skin tannin extract (SkinT16.5) (on gelatin dry basis). Slight differences in thickness are related to small variations in film inclination grades, which led to different apparent thicknesses (scale bars: 20 µm (top row a)–c) × 800 magnification), 5 µm (bottom row a′)–c′)) × 4000 magnification).

Table 3
Solubility values of gelatin films after immersion in Milli-Q water (MQW) and 50 % v/v ethanol (50EtOH) for 24 h at 4 and 22 °C; and release and inhibition values of gelatin films after immersion in 50EtOH for 24 h at 4 °C, as a function of seed tannin (SeedT) concentrations (1 % & 2 %) and skin tannin (SkinT) concentrations (11 % & 16.5 %) (on a gelatin dry weight basis). Two means followed by the same letter in the same column are not significantly different ($p > 0.05$) through Tukey's multiple range test.

	Solubility (%)				Release (%)	
Films	MQW (4 °C)	MQW (22 °C)	50EtOH (4 °C)	50EtOH (22 °C)	50EtOH (4 °C)	Inhibition[a] (%)
Control	2.4 ± 0.3^{abc}	100^{a}	2.2 ± 0.7^{a}	29.7 ± 5.7^{a}	–	–
SeeT1	3.3 ± 0.5^{bc}	100^{a}	2.8 ± 0.7^{a}	32.1 ± 0.4^{a}	9.5 ± 0.6^{a}	6.0 ± 1.1^{a}
SeedT2	1.6 ± 0.5^{a}	100^{a}	2.2 ± 0.8^{a}	28.3 ± 1.7^{a}	13.9 ± 1.9^{b}	13.3 ± 1.6^{b}
SkinT11	2.1 ± 0^{ab}	100^{a}	2.3 ± 0.9^{a}	35.9 ± 6.7^{a}	20.4 ± 2.1^{c}	3.5 ± 0.5^{c}
SkinT16.5	3.7 ± 0.6^{c}	100^{a}	3.1 ± 0.6^{a}	33.2 ± 0.6^{a}	18.9 ± 0.8^{c}	5.4 ± 0.6^{ac}

[a] Inhibition values of released tannin extracts in 50EtOH solution diluted (25/1000) in 75 μM DPPH solution.

Kilmartin, et al., 2021). Therefore, the release of extracts, their antioxidant activity and colour retention of films were analysed in 50EtOH-4 °C storage conditions (Table 3 and Fig. 6).

The extract-containing films showed a burst of release in the first hour, followed by a slower sustained release up to 2 h, ultimately reaching a plateau (Fig. 6 **a**). The same release behaviours were observed, irrespective of extract type and concentration. However, unlike SkinT, the release of SeedT extract from films was concentration-dependent, since more SeedT was released as the extract concentration increased. The SkinT11 & SkinT16.5 extracts showed higher release values (20.4 ± 2.1 % and 18.9 ± 0.8 %, respectively) after 24 h of immersion, compared to the SeedT1 and SeedT2 extracts (9.5 ± 0.6 % and 13.9 ± 1.9 %, respectively). This could be related to higher amounts of phenolic compounds in the SeedT extract that could induce further interactions with gelatin, as suggested from the WCA and FTIR results, which could retain the SeedT extract for a longer period of time in the films.

As the extracts presented antioxidant properties (Table S2), the inhibition activity of the released extracts in 50EtOH was also analysed (Table 3). Although the release of tannin extracts from SeedT films was 2 times lower than from SkinT films, SeedT films had similar (SeedT1) and double (SeedT2) inhibition capacity compared to the SkinT films. This was related to a higher content of phenolic compounds in SeedT extract than in SkinT extract (Tables S1 and S2). The addition of grape tannin extracts in the packaging material, especially the SeedT extract, their migration from the packaging to the food simulant, and their primary antioxidant activity (reaction with free radicals) can be considered as active functions for food packaging applications (e.g., to reduce the lipid oxidation on the sliced dried-cured coppa (meat) (Luciano et al., 2022)).

The release of the extract from the films could also affect the colour properties of films since films may lose their colour and consequently become imperceptible to the naked eye, as seen in the literature (Etxabide, Kilmartin, et al., 2021). The immersion of films in 50EtOH solution did not have a notable effect on the colour properties of films, since insignificant changes were observed in the colour parameters (L*, a*, b*, and ΔE*) of films before and after immersion (Fig. 6 **b**), and films continued being perceptible. This could be related to the low release percentage of extracts in films (< 20 %), as seen in Fig. 6 **a**.

3.2.5. *Colour response analysis of the films*

pH variations in food can be sensed via the interaction of films with compounds on the food surface (direct contact of film with food), or via volatile compounds released from the food and retained in the headspace of the packaging (indirect contact). Therefore, the pH indicator ability of films containing tannin extracts was analysed by direct (damped with NaOH) and indirect (release of ammonia from NH_4OH) pH alterations, as can be seen in Fig. 7 **a** and **b**, respectively. Unlike SeedT, the addition of SkinT into the films provided a sensing capacity with predictable colour differences ($\Delta E^* \geq 3$), either to direct or indirect pH alterations (Fig. 7 **c** and **d**) due to the molecular structural transformations of the polymeric pigments present, as seen in Fig. 1 **b** and **f**. The pH alterations were immediately sensed when the films were in direct contact with alkaline surfaces (Fig. 7 **c**), while predictable colour changes in SkinT films happened after the films were exposed to alkaline environments for a minimum of 30 min (Fig. 7 **d**).

Regarding SeedT films, some colour changes were observed, mainly in films with higher extract content (SeedT2, Fig. 7 **a**). However, these colour differences were considered small ($\Delta E^* \leq 2$) and fairly ($\Delta E^* \leq 3$) predictable (Paciulli et al., 2017) for SeedT1 and SeedT2 films, respectively, irrespective of direct/indirect pH alterations and exposure times

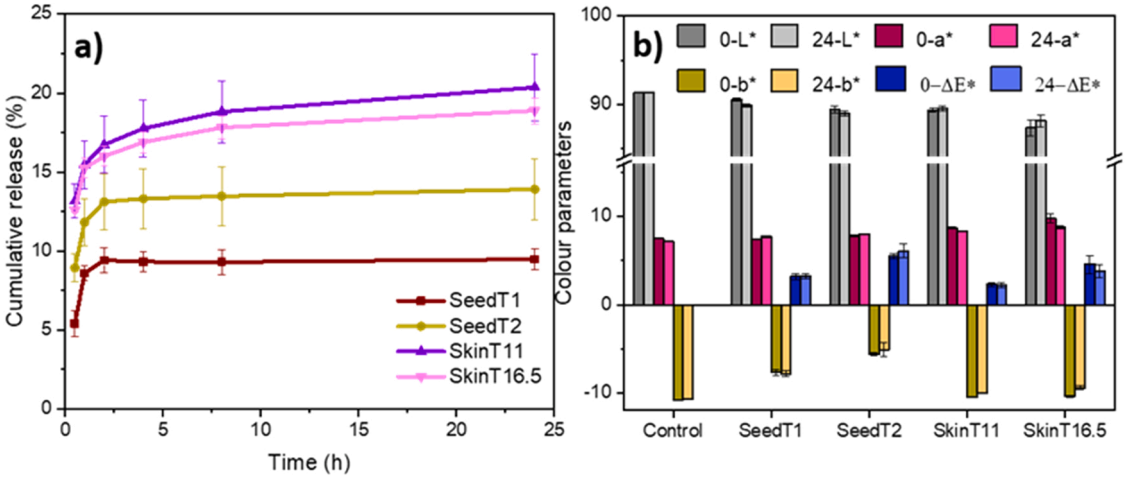

Fig. 6. a) Release of seed tannin (SeedT) and skin tannin (SkinT) extracts from gelatin films as a function of concentration (1 % & 2 % and 11 % & 16.5 %, respectively) and time; b) gelatin films colour parameters (L*, a*, b* and ΔE*) values before (0-) and after immersion in 50EtOH at 4 °C for 24 h (24-).

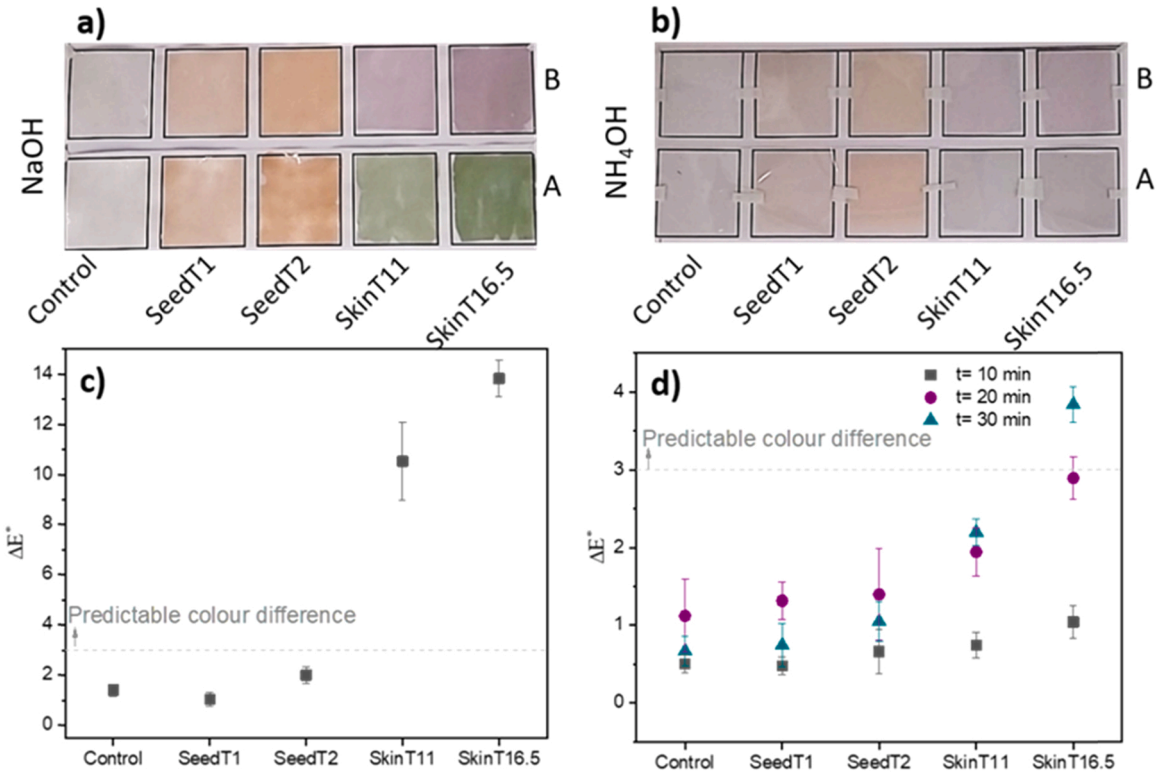

Fig. 7. Colour change of gelatin films with 1 % and 2 % seed tannin (SeedT1 & SeedT2) and 11 % and 16.5 % skin tannin (SkinT11 & SkinT16.5) extracts while a) direct and b) indirect pH variations. B and A mean before and after, respectively; Total colour difference (ΔE*) of films c) after direct pH variations and d) during indirect pH variations (up to 30 min).

(Fig. 7 **c** and **d**). These colour sensing variations can be related to the addition of different concentrations of both extracts, as well as diverse interactions among the gelatin and extracts that either allowed or hindered the molecular transformation of SkinT and SeedT extracts, respectively. It was seen (Fig. 3 **and** 6**;** Table 2) that the addition of the SeedT extract could induce higher interactions with gelatin that could impede its molecular transformations in films. It is worth mentioning that colour changes induced in SkinT films by direct pH alterations did not show any colour reversibility, however, indirect pH alterations were reversible. This could be due to the retention of sodium salts in the damped films after drying, as well as the capacity of gelatin to absorb and desorb moisture (Yang et al., 2020), which induced polymeric pigment molecule transformations. The reversibility of colour observed in SkinT films after indirect pH alterations could be a disadvantage when the food package is open, and the inner atmosphere of the packaging changes. This can lead to inaccuracies in predicting the freshness of the food.

4. Conclusions

The grape seed tannin (SeedT) extracts presented higher phenolic content and thus higher antioxidant activity than the grape skin tannin (SkinT) extracts, while both extracts showed colour and intensity variations as a function of solution pH. The addition of different concentrations of extracts into gelatin formulations produced coloured and transparent films with enhanced UV light absorption capacity, which provided films with a preventive antioxidant (active) function. The wettability of films decreased, which was related to interactions such as hydrogen bonding between gelatin and phenolic compounds in the extracts, as seen in the shift and broadening of the amide A band in FTIR results. These enhancements were more notable as the SeedT concentration increased (from 1 % to 2 %), while no significant changes were observed as the amount of SkinT increased (from 11 % to 16.5 %). The immersion of films in 50 % ethanol at 4 °C resulted in the release of tannin extracts with antioxidant activity, and the SeedT extract showed higher activity which was concentration-dependent. The release of extract did not affect the colour properties of the films. The direct and indirect pH modifications of the films showed that SkinT films were able to sense and display predictable colour differences (intelligent function), while no significant colour changes were observed with the SeedT films. This was related to more physical interactions between the gelatin and phenolic compounds, which could hinder the molecular transformation of compounds in SeedT. The SeedT-containing films were more suitable for active (primary and secondary antioxidant) packaging, while the addition of SkinT extract could be used to develop active (preventive antioxidant function) as well as intelligent (pH sensing) packaging for chilled foodstuffs, such as seafood and meat products whose pH increases to basic values as a result of spoilage, due to how these foodstuffs release chemicals (e.g., volatile amines) that increase the pH of the food during food decomposition.

CRediT authorship contribution statement

Alaitz Etxabide: Conceptualization, Methodology, Validation, Investigation, Writing – original draft, Visualization, Writing – review & editing. **Yi Yang:** Investigation, Writing – review & editing. **Juan I. Maté:** Writing – review & editing, Supervision. **Koro de la Caba:** Writing – review & editing, Funding acquisition, Supervision. **Paul A. Kilmartin:** Resources, Writing – review & editing, Funding acquisition, Supervision.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2022.100896.

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