Effect of curcumin, betanin and anthocyanin containing colourants addition on gelatin films properties for intelligent films development

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Abstract

About 52 Mt of food waste is generated annually at the household level. Almost a quarter of this waste is related to packaging issues such as inaccurate data labelling systems. This shows the crucial need to replace or improve food data labels in order to reduce food waste. Gelatin films with different concentrations of curcumin, betanin and anthocyanin containing colourants were prepared by casting. The effect of different concentrations of colourants on the optical, barrier, physicochemical, structural and colour response properties of films was investigated. The addition of colourants altered the colour, light barrier, and wettability of the films. Although the colourant retention capacity of films varied as a function of colourant type, no changes were observed in infrared spectra, and likewise the similar results were obtained by X-ray diffraction, mechanical testing and scanning electron microscopy pointed to retention of tertiary and inner structural properties of the films. The addition of colourants provided films with the capacity to sense pH changes before and after immersion in a fatty food simulant. These properties establish the suitability of the films for intelligent fatty food packaging applications.

Keywords: gelatin, curcumin, betanin, anthocyanin, intelligent films, pH indicator.

1. Introduction

Food waste is a major global challenge from social, environmental and economic points of view. It represents an inefficient use of the scarce resources used to produce it, such as land and water, as well as the unnecessary greenhouse gas emissions that contribute to global warming and climate change (FAO, 2011). Along the food supply chain in the European Union, the largest food waste is generated at the household level which represents almost 40% of the total (129.2 Mt) food waste, (Caldeira, De Laurentiis, Corrado, van Holsteijn, & Sala, 2019). About 20-25% of household food waste is related to packaging characteristics (Williams, Wikström, Otterbring, Löfgren, & Gustafsson, 2012), such as date labelling systems (European Commission, 2018). In fact, current data labelling does not provide consumers with real-time information about the freshness, safety and quality of food products, and consequently, huge amounts of wholesome edible food are wasted. This reveals the crucial need to replace or improve food data labels in order to reduce food waste.

In this regard, intelligent packaging systems include materials that allow the condition of packaged food or the environment surrounding the food to be monitored (Regulation (EC) N^{o} 1935/2004). These materials include indicators, substances that determine the presence or absence of a component, the concentration of a particular substance, and can involve reactions between compounds (Yam, 2012; Yousefi et al., 2019). They can provide customers with direct, visual and *in situ* information about the quality and freshness of foodstuffs. In place of an arbitrary expiration date on the product package, as with current labels, intelligent packaging systems can lessen the need to dispose of large amounts of wholesome food products.

The information provided by the indicators is the result of reactions between substances formed from biological, chemical and physical processes within food. These include, as an example, the formation of volatile organic compounds derived from microbial growth, such as acetic acid and amines, which interact with the integrated indicators within the package. These

changes are closely correlated with alterations in the pH of the food, which in turn is related to food spoilage. Therefore, the addition of pH indicators into food packaging systems could dynamically sense changes in food (Han, Ruiz-Garcia, Qian, & Yang, 2018), and provide a visual message, such as a change of colour of the indicator, to the consumers about the quality of the food. In this respect, and considering the changes in consumer preferences toward natural and non-toxic substances over synthetic chemicals, natural and food grade colourants should be used when the packaging is in contact with foodstuffs. In this matter, curcumin, betanin, anthocyanins and chlorophylls, among others, are food grade colourants which have shown colour sensitivity to pH, and so they can be considered as suitable indicators for the development of intelligent packaging systems (Latos-Brozio & Masek, 2020). These indicators may be incorporated into packaging materials.

Regarding the packaging material, the amount of single-use synthetic packaging consumption is increasing globally and so are the environmental impacts that it entails. To achieve sustainability in food packaging, new packaging innovations based on renewable resources have been studied in order to develop sustainable or green food packaging materials. Biodegradable and edible materials derived from proteins, such as fish gelatin, have shown potential to be used in food packaging due to their abundance, renewability, film forming ability, transparency, excellent barrier properties against O_2, CO_2 and lipids. They are also excellent vehicles for incorporating a wide variety of additives (Etxabide, Uranga, Guerrero, & de la Caba, 2017) and showed no allergenicity risks at the doses typically used in food products (Hansen et al., 2004).

The aim of this study was to develop and characterize colorimetric indicator intelligent food packaging films based on gelatin incorporated with different concentrations of curcumin, betantin, and anthocyanin containing natural food colourants. The effect of the colourants and their concentration on colour, UV-vis light barrier, transparency, water contact angle and the

solubility properties of the films were assessed. Furthermore, interactions between the components, as well as the inner structure of the films, colour retention and colour change capacity to external pH stimuli, were examined in order to study the suitability of the colourants, their concentrations and compatibility with gelatin for intelligent food packaging applications.

2. Materials and methods

2.1 Materials

A commercial cod fish gelatin type A (Weishardt International, Liptovsky Mikulas, Slovakia) was employed as a biopolymer matrix to form films. This gelatin has a bloom value of 200, 11.06 % moisture and meets the quality standard for edible gelatin (1999/724/CE). Commercial food colourants were used as pH indicator additives and were supplied by Chr Hansen. The following information was provided by the company: T-PT8-WS is a dark yellow to brown viscous liquid produced by the extraction of pigments from turmeric root (*Curcuma longa* L.), with curcumin as the major colouring principle, while polysorbate 80, an authorized food additive (classification number E-433), is present as a non-ionic emulsifier (Chr. Hansen Natural Colors A/S, Denmark); B-50-WS is a dark purple liquid produced by the extraction of pigments from red beet (*Beta vulgaris* L.) and its subsequent pasteurization, with betanin as the major colouring principle and citric acid the acidity regulator (Chr. Hansen Natural Colors A/S, Denmark); ColorFruit Carrot 9 WS is a dark red liquid, produced by the extraction of pigments from black carrots (*Dacus carota* L.) and its subsequent pasteurization, with anthocyanins as the major colouring principle and citric acid the acidity regulator (Chr. Hansen Italia SpA, Italy). All colourants were water soluble, natural, and food grade. The possible presence of other chemical compounds could have some effect on the final properties of films.

In the European Union, curcumin, betanin, and anthocyanin-derived colourants are categorized under classification numbers E-100, E-162 and E-163, respectively (Commission

111 Regulation (EU) N^{o} 1129/2011). These numbers have been used to label the film containing
112 colourants in this study.

113 **2.2 Film preparation**

114 Fish gelatin-based films with different colourants contents were prepared by casting. 2.5
115 g of gelatin was dissolved in 40 mL deionized water for 30 min at 65 °C under continuous stirring
116 (250 rpm) to obtain a good blend of the film forming solution (FFS). After that, the FFS was left
117 to cool down to 26 °C and the required amount of colourant in 10 mL deionized water (colourant
118 absorbance values of 0.50, 1.00 and 2.00 in the final volume of FFS, 50 mL) was added into FFS
119 which was then mixed at 250 rpm for 5 min. The final gelatin concentration on the FFS was 5
120 w/v% (Stevenson et al., 2020). Finally, 16.00 g FFS was poured into each Petri dish (ø 150 mm)
121 and left them to dry and form the film in an air-circulating fume hood at 20 °C and 56% RH for
122 24 h. The samples were then peeled from the dishes and named as E-100A0.5, E-100A1, E-
123 100A2; E-162A0.5, E-162A1, E-162A2; and E-163A0.5, E-163A1, E-163A2 for E-100, E-162 and E-
124 163 colourants solutions with initial absorbance values of 0.5, 1.0 and 2.0, respectively. Films
125 without colourants were prepared as control samples. All samples were protected from light
126 during storage at 20 °C and 63% RH.

127 The absorbances of colourants solutions in deionized water (10 mL) were analysed by
128 UV-vis spectrophotometry (Shimadzu UV-3600plus, Shimadzu Europa, GmbH, Germany) and the
129 software UVProve Version 2.5 in the visible spectrum (300-800 nm), to verify each colourant
130 intensity values before mixing with the gelatin solutions. The wavelengths of maximum
131 absorbance (λ_{max}) and calibration curves of colourants were previously obtained using UV–Vis
132 spectroscopy: λ_{max} of 424, 532, 528 nm for E-100, E-162 and E-163, respectively and calibration
133 curves for E-100 ($y = -0.012 + 10.708x$, $R^2 = 0.999$), E-162 ($y= 0.010 + 0.488x$, $R^2= 0.999$) and E-
134 163 ($y= -0.042 + 0.565x$, $R^2= 0.999$) at each colourant λ_{max}. It is worth mentioning that calibration
135 curve equations showed E-100 was a more powerful colourant than E-162 and E-163 since much

lower concentrations (almost 20 times less) were required to obtain the same absorbance values (colour intensity).

2.3 Film characterization

2.3.1 Colour measurement

Colour was determined with the colorimeter 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* 91.49, a*6.65 and b* -9.85) 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 colourants, as a function of colourant concentration, were calculated referred to the control films (placed onto a white paper sheet) as follows:

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

2.3.2 UV–vis absorption and transparency measurements

A Shimadzu UV-3600plus spectrophotometer (Shimadzu Europa, GmbH, Germany), and the software UVProve Version 2.5, were used to assess the effect of the addition of different colourants concentrations on the barrier properties of films to ultraviolet (UV) and visible (vis) light at wavelengths from 200 to 800 nm. In addition, the transparency of the films was determined at 600 nm. Three specimens were tested for each composition (n=3) and the transparency (T) was calculated as follows:

$$T = \frac{A_{600}}{x}$$

where A_{600} is the absorbance value at 600 nm and *x* is the average thickness (mm) of the films. Film thickness was measured using a digimatic micrometre (ID-H Series 543 Mitutoyo, China) and six measurements at different positions were taken on each film. Higher values of the

transparency indicate a lower transparency for the films (Garrido, Etxabide, Guerrero, & de la Caba, 2016).

2.3.3 Water contact angle (WCA) determination

WCA measurements were performed using an optical contact angle measuring instrument (CAM 100, KSC instruments Ltd., Finland). A 4 µL droplet of deionized water was placed on different sample surface regions to estimate the hydrophilic/hydrophobic character of films. The side (air-facing and the plate-facing) of films was chosen randomly. The image of the drop was taking using CAM100 software after 5 s of the droplet deposition. Ten measurements (n=10) were taken for each composition at 20 °C and 67% RH.

2.3.4 Solubility

Three specimens (n=3) of each film were weighed (W_o) and immersed in 4 mL of a 50% ethanol (50EtOH) solutions. The flasks were wrapped in aluminium foil and stored in a fridge at 4 °C for 48 h. After that, specimens were taken out of the food simulant 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 by the following equation:

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

2.3.5 Colour retention capacity of films

The capacity of films to retain the colour was measured using a Shimadzu UV-3600plus spectrophotometer and the software UVProve Version 2.5. Films (1 x 4 cm^2) were immersed in 4 mL of 50EtOH solution, and the flasks, wrapped in aluminium foil, were stored in a fridge at 4 °C. At particular time intervals (1, 4, 8, 24 and 48 h), the absorption spectra of the solutions was recorded from 300 to 650 nm. Then, films were taken out of 50EtOH and left to dry in an air-circulating fume hood at room temperature (20 °C) for 24 h before measuring their UV-Vis light absorption capacity from 250 to 650 nm. All tests were carried out in triplicate (n=3).

184 **2.3.6 Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy**

185 FTIR spectra of the individual components and films were carried out on a Bruker Vertex
186 70 FTIR spectrometer using a single bounce Platinum Diamond Micro-ATR accessory (Bruker
187 Optics, New Zealand) in order to analyse the interactions between the colourants and gelatin.
188 The raw food colourants were frozen (-29 °C for 24 h), and then freeze-dried (Labconco-12Plus,
189 0.2 Torr, condenser temperature -85 °C for 24 h) before their characterization. A total of 32
190 scans were performed at 4 cm^{-1} resolution and the measurements were recorded between 4000
191 and 800 cm^{-1}. Normalization of each obtained spectra were done using OPUS 7.5 software.

192 **2.3.7 X-ray diffraction (XRD)**

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

198 **2.3.8 Mechanical properties**

199 Tensile strength (TS) and elongation at break (EB) were measured for each film at least
200 five times on an Instron 5943 mechanical testing system (Instron, Norwood, USA) according to
201 ASTM D882-02 (ASTM, 2002). Samples were cut into strips of 50 mm x 10 mm, the gauge length
202 was set to 30 mm, and the testing was carried out at 1 mm/min crosshead speed with a 50 N
203 load cell.

204 **2.3.9 Scanning electron microscope (SEM)**

205 Cryofractured samples were fixed on glass slides using carbon tapes and sputter-coated
206 with gold for 2 minutes using a sputter coater (Quorum Q150R S, Australia). SEM (JCM-6000
207 Versatile Benchtop SEM, Korea) was used to observe the cross section morphology of films at
208 an accelerating voltage of 10 kV and a magnification of 800 x.

2.3.10 Colour response analysis of films

Films (2 x 2 cm^2) were deposited onto filter paper sheets (2 x 10 cm^2) previously damped with 300 µL of different solutions (deionized water (pH ≈7), 0.5 M acetic acid (AAc, pH ≈3), pH 4 and pH 10 buffers, and 0.5 M NaOH (pH ≈14)) in order to observe with the naked eye the colour response of films containing different concentrations of colourants to various pH containing surfaces and colour stability after film drying in an air-circulating fume hood at room temperature (20 °C) for 24 h . Films with no colourants were use as controls. In addition, the colour response of E-100A2, E-162A2 and E-163A2 films to pH 10 buffer after conducting the colour retention test was also analysed. All tests were carried out in triplicate (n=3).

2.4 Statistical analysis

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

3. Results and discussion

3.1 Optical and barrier properties of films

Colour is relevant since it directly influences product appearance and thus, consumer acceptability (Monedero, Fabra, Talens, & Chiralt, 2009). As shown in **Figure 1**, films were homogenous and transparent, and the colour changed with the addition of colourants.

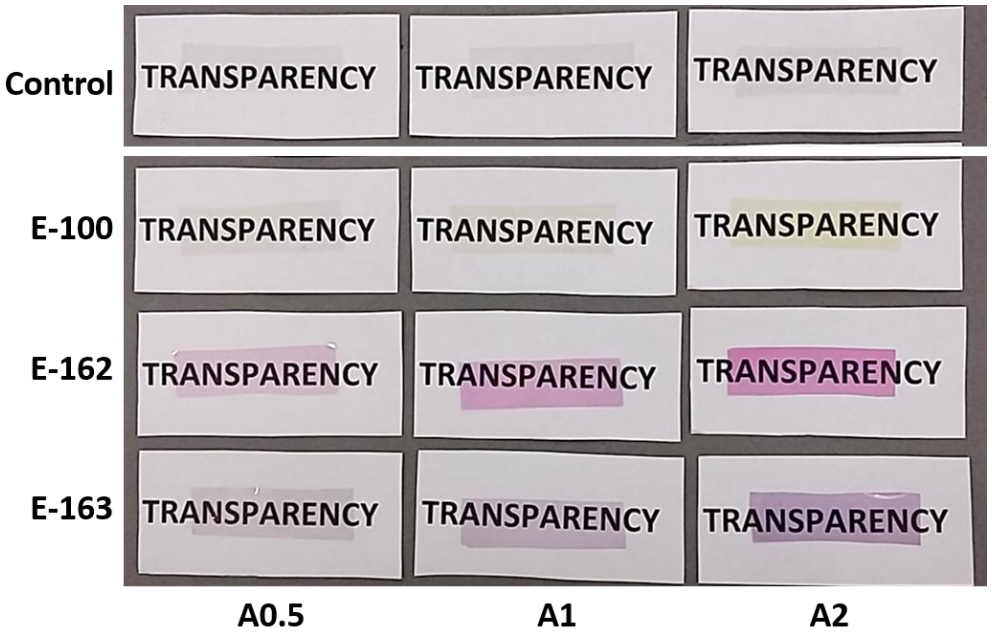

Figure 1. Gelatin-based films with different concentrations (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163).

The presence of colourants in the films caused a decrease in L* values (lightness reduction) (**Table 1**). Regarding the a* parameter, these values significantly decreased (toward greenness) and increased (toward redness), while the b* parameter notably increased (toward yellowness) and decreased (toward blueness), when E-100 and E-162/E-163 were added, respectively. The effects were greater when higher colourant concentrations were used, irrespective of colourant type. These colour variations were related to the yellow (λ_{max} 424 nm), purple (λ_{max} 532 nm) and red (λ_{max} 528 nm) colour characteristics of E-100, E-162 and E-163 natural colourants, respectively (Zumdahl & DeCoste, 2013). Therefore, the total colour difference (ΔE*) increased with colourant addition into the films. However, the E-100 films showed the lowest ΔE* values followed by E-163, which presented slightly larger values than the E-100 films, and by E-162 which displayed by far the largest ΔE*.

Protection against light also plays a critical role in food packaging, since light can lead to vitamin degradation, discolouration of fresh food, development of off-flavours and auto-oxidation of fats (Duncan & Chang, 2012). As can be observed in **Figure 2**, all of the gelatin films

exhibited high protection against UV light (200-300 nm) related to the presence of COOH, $CONH_2$ and C=O groups in polypeptide chains and peptide bond of cod fish gelatin (200–250 nm), as well as chromophores such as tyrosine and phenylalanine (250–300 nm), common aromatic amino acids found in proteins (Bonilla & Sobral, 2016; Etxabide, Uranga, Guerrero, & de la Caba, 2015). Therefore, gelatin films could potentially provide light protection and preserve the quality of food.

Table 1. Colour parameter (L*, a*, b*, ΔE*), transparency and thickness values of gelatin films as a function of colourants (E-100, E-162 and E-163) and their concentrations (initial absorbance (A) 0.5, 1 and 2). Two means followed by the same number/letter in the same row are not significantly (P > 0.05) different through the Tukey's multiple range test.

Film	L*	a*	b*	ΔE*	Transparency	Thickness (μm)
Control	90.89 ± 0.13 $^{1/ab/AB}$	6.48 ± 0.08 $^{1/a/A}$	-9.17 ± 0.12 $^{1/a/A}$	-	1.00 ± 0.05 $^{1/a/A}$	48.8 ± 5.3$^{1/a/A}$
E-100A0.5	90.85 ± 0.09^{1}	4.38 ± 0.05 ^{1}	-4.11 ± 0.10^{1}	5.48 ± 0.10^{1}	0.97 ± 0.04^{1}	44.3 ± 9.9^{1}
E-100A1	90.82 ± 0.13^{1}	3.44 ± 0.09 ^{1,2}	-1.94 ± 0.24^{1}	7.84 ± 0.23^{2}	0.92 ± 0.01^{1}	45.1 ± 4.2^{1}
E-100A2	90.57 ± 0.09^{1}	0.91 ± 0.11^{2}	4.36 ± 0.11^{2}	14.64 ± 0.06^{3}	0.82 ± 0.01^{2}	48.7 ± 6.0^{1}
E-162A0.5	87.49 ± 0.14^{a}	12.63 ± 0.18^{a}	-11.29 ± 0.12^{a}	7.34 ± 0.25^{a}	1.08 ± 0.05^{a}	48.0 ± 8.0^{a}
E-162A1	84.75 ± 0.07^{ab}	18.96 ± 0.29^{b}	-12.92 ± 0.02^{a}	14.4 ± 0.24^{b}	1.19 ± 0.01^{a}	54.4 ± 3.7^{a}
E-162A2	79.48 ± 0.24^{b}	26.65 ± 0.67^{c}	-14.34 ± 0.06^{a}	23.74 ± 0.51^{c}	1.78 ± 0.14^{b}	51.6 ± 5.5^{a}
E-163A0.5	87.36 ± 0.15^{A}	9.37 ± 0.10^{AB}	-10.77± 0.12^{AB}	4.83 ± 0.21^{A}	1.43 ± 0.10^{A}	44.7 ± 5.5^{A}
E-163A1	84.45 ± 0.39^{AB}	11.92 ± 0.23^{B}	-11.95 ± 0.06^{B}	8.88 ± 0.40^{B}	2.15 ± 0.14^{B}	44.3 ± 3.8^{A}
E-163A2	78.61 ± 0.31^{B}	16.66 ± 0.46^{C}	-14.35 ± 0.28^{B}	16.78 ± 0.39^{C}	3.05 ± 0.33^{C}	47.2 ± 5.3^{A}

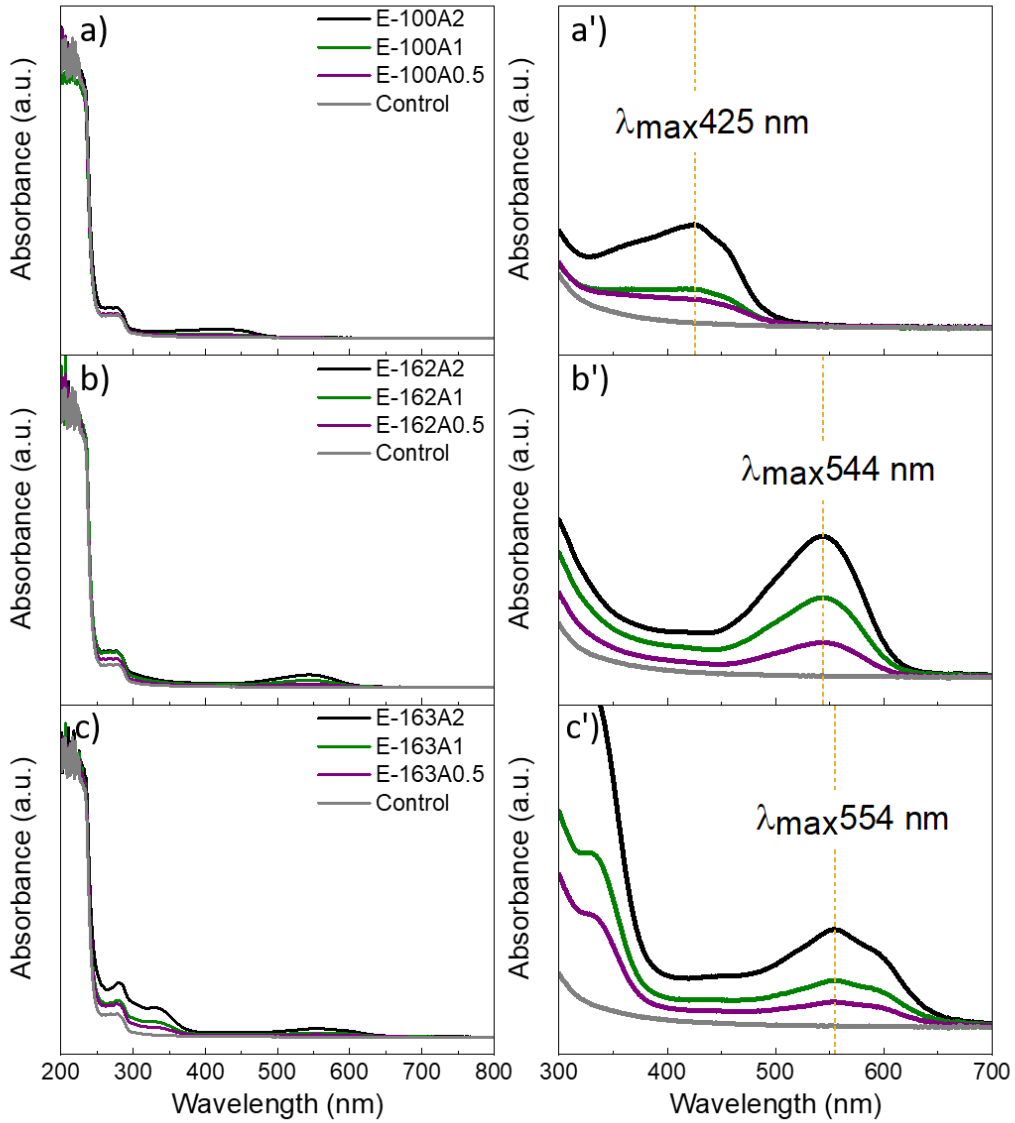

Figure 2. UV-Vis spectra for gelatin-based films with different concentrations (initial absorbance (A) 0.5, 1 and 2) of added colourants a) E-100, b) E-162 and c) E-163 and their magnifications a'), b') and c'), respectively.

When colourants were added, an improvement of light absorbance capacity of films in the visible spectrum (λ_{max} 425, 544 and 554 nm, respectively) was observed, showing major enhancements with the increase of colourant concentrations, irrespective of colourant type. In addition to that, E-163 films presented an improvement in UV region which could be related to

the presence of other uncoloured compounds in the product that absorb UV light between 250-300 nm.

It is worth noting that, although FFSs were prepared starting from the same colour intensities (initial maximum absorbance (A_{max}) values of 0.5, 1 and 2), different A_{max} values were observed in films. This could be because the colour and intensity of films are directly influenced by the type of the colourant and the interactions between the colourant and the matrix (Atares & Chiralt, 2016). Films containing E-100 and E-163 colourants presented similar and lower A_{max} values than E-162 films, although similar colourant concentrations were used for E-162 and E-163 film development. In addition to that, A_{max} values of films containing E-162 and E-163 were at a higher wavelength in the visible spectrum (λ_{max} 544 and 554 nm) than observed in deionized water (λ_{max} 532 and 528 nm), and in the gelatin FFSs (λ_{max} 534 and 543 nm), while E-100 remained unchanged (λ_{max} 424 nm). These bathochromic shifts and intensity variations in the visible region could be related to molecular structural transformations in colourants as well as to possible weak interactions (non-covalent interactions) of colourants with gelatin, during the film preparation process which could alter the matrix network (Chatterjee & Kumar, 2016; Kennedy & Waterhouse, 2020; Yang, Yang, Fan, Li, & Hou, 2018; Zumdahl & DeCoste, 2013). Consequently, these changes had a direct effect on the final colour properties of films, and so on ΔE* values previously observed.

Transparency is also an important characteristic of the films, since it enables direct visual inspection of the quality and freshness of the product. Unlike E-100, the addition of E-162 and E-163 colourants notably reduced the transparency of the films (**Table 1**), especially the latter colourant, and larger decreases were observed when higher colourant concentrations were used. These reductions could be related to the E-162 and E-163 colourant molecules, which absorb light in the yellow-green region of the visible spectrum, and thereby hindered the transmission of the visible light through the films (Mir, Dar, Wani, & Shah, 2018). The

transparency lowering could also be connected to the decrease in lightness (L*) previously observed in colour assessment (Pereira Jr., Queiroz de Arruda, & Stefani, 2015). E-100 films showed an improvement of transparency at the highest colourant concentration. This could be related to more polysorbate 80 being present, since being a surfactant, emulsifier and thickener, it could enhance the solubilisation of the E-100 colourant in the gelatin films. On the whole, the films remain transparent (**Figure 1**).

Surface properties of food packaging materials, such as wettability, are important not only to control the quality, prolong the shelf-life, and maintain the appearance of packaged food (Han, Zhang, & Buffo, 2005) but also to permit food components to maintain contact with the packaging material surface, a key property for intelligent packaging development. In order to determine the hydrophilic or hydrophobic character of the film surface, WCA values were measured. As can be seen in **Figure 3**, the addition of E-100 notably increased the hydrophilic character of the films, mainly at higher E-100 concentrations. Although it has been reported that the addition of curcumin improved the hydrophobicity of bio-based films, due to its hydrophobic nature (Ezati & Rhim, 2020), the major presence of polysorbate 80, a hydrophilic and non-ionic surfactant, considerably increased the wettability of the gelatin films. As for E-162 and E-163, the addition of the colourants led to a notable decrease in hydrophilicity of the film surface. This could be related to possible interactions between the colourants and gelatin via hydrogen bonding, which could reduce the exposure of hydrophilic groups at the film surface and their interactions with water molecules (Mir et al., 2018). With these films, the increase in colourants concentration had no notable effect on WCA values.

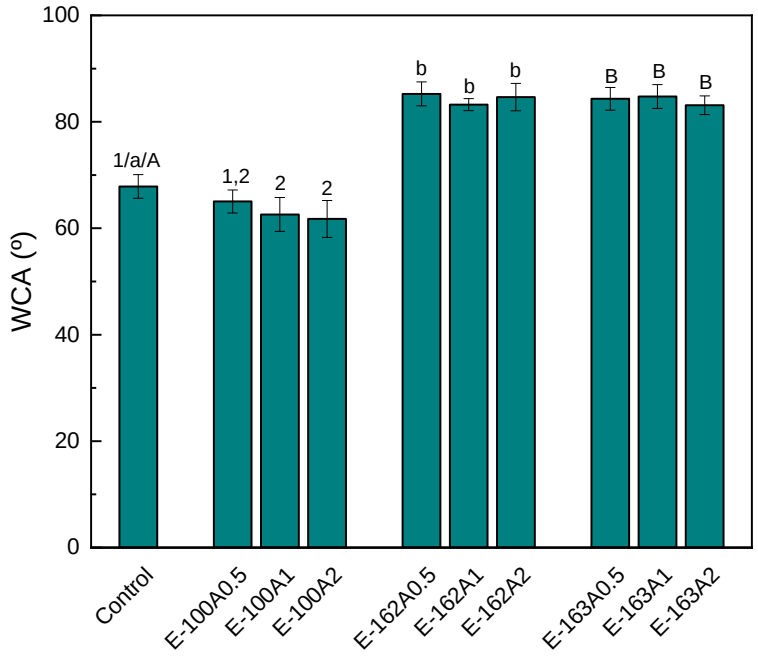

Figure 3. Water contact angle (WCA) values of gelatin films with different concentrations (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163). Two means followed by the same number/letter are not significantly ($P > 0.05$) different through the Tukey's multiple range test. (n=10)

3.2 Physicochemical and structural properties of films

Gelatin is a hygroscopic protein, and gelatin coming from cold-water fishes such as cod, present low melting point temperatures (Karim & Bhat, 2009). Therefore, solubility was studied in the 50EtOH food simulant (replication of oil/water mixtures, Commission regulation (EU) 2016/1416) under chilled storage conditions, so that the films could retain their physical integrity (thickness, area, size) without affecting the measurement of physical properties. As can be seen in **Table 2**, control films presented 4.8 % solubility, which was solely related to gelatin release into the food simulant. When colourants were added, the larger the colourant percentage in the films, the higher the solubility of the films, especially with the E-162 and E-163 samples. In fact, these last films showed higher solubility than the E-100 samples, since in

E-100 films, lower amounts of colourant were used because of its higher colouring power. Higher film solubility values could also be related to a decrease in gelatin stability due to the presence of E-162 and E-163 colourants. However, these values can be considered as low solubility values (Yoshida, Maciel, Mendonça, & Franco, 2014). These results could also indicate that, along with gelatin, the colourants might be released during film immersion in 50EtOH.

Table 2. Colourant percentage (in gelatin dry basis) and solubility values of gelatin films with different concentrations (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163). Two means followed by the same number/letter in the same row are not significantly (P > 0.05) different through the Tukey's multiple range test. (n=3)

Film	Colourant (%)*	Solubility (%)
Control	-	$4.8 \pm 0.4^{1/a/A}$
E-100A0.5	0.05	4.5 ± 0.3^{1}
E-100A1	0.09	4.3 ± 0.3^{1}
E-100A2	0.19	4.8 ± 0.3^{1}
E-162A0.5	1.00	5.9 ± 0.7^{ab}
E-162A1	2.03	6.2 ± 0.3^{b}
E-162A2	4.08	8.0 ± 0.2^{c}
E-163A0.5	0.96	5.7 ± 0.6^{AB}
E-163A1	1.85	5.2 ± 0.4^{A}
E-163A2	3.62	6.5 ± 0.4^{B}

*on gelatin dry basis

In order to analyse the colourants release behaviour into food simulants, the colour retention capacity of films was studied. When film were immersed in 50EtOH for 2 days, gelatin (**Figure 4-II a**) and colourants (**Figure 4-II b-d**) were released from the films into the food simulant, and more so when more colorants were added into the films, irrespective of colourant type. The colourants were mainly released over the first hour of immersion, and the release continued to increase gradually with time, but with no difference between 24 and 48 h of immersion. Unlike E-100 and E-163, films containing E-162 showed almost no release of colourant in 50EtOH. This could be due to interactions between the colourant and the gelatin and/or the steric hindrance effects on the release of the colourant. Consequently, E-162 showed

better colour retention capacity compared to E-100 and E-163 films, as seen in the small coloration of the 50EtOH solution after 48 h of film immersion (**Figure 4-I**). It is worth mentioning that although the E-162 film presented the lowest release of the colourant into 50EtOH, these films showed the largest solubility values. This can be related to release of gelatin and/or other uncoloured and unknown compounds from the films.

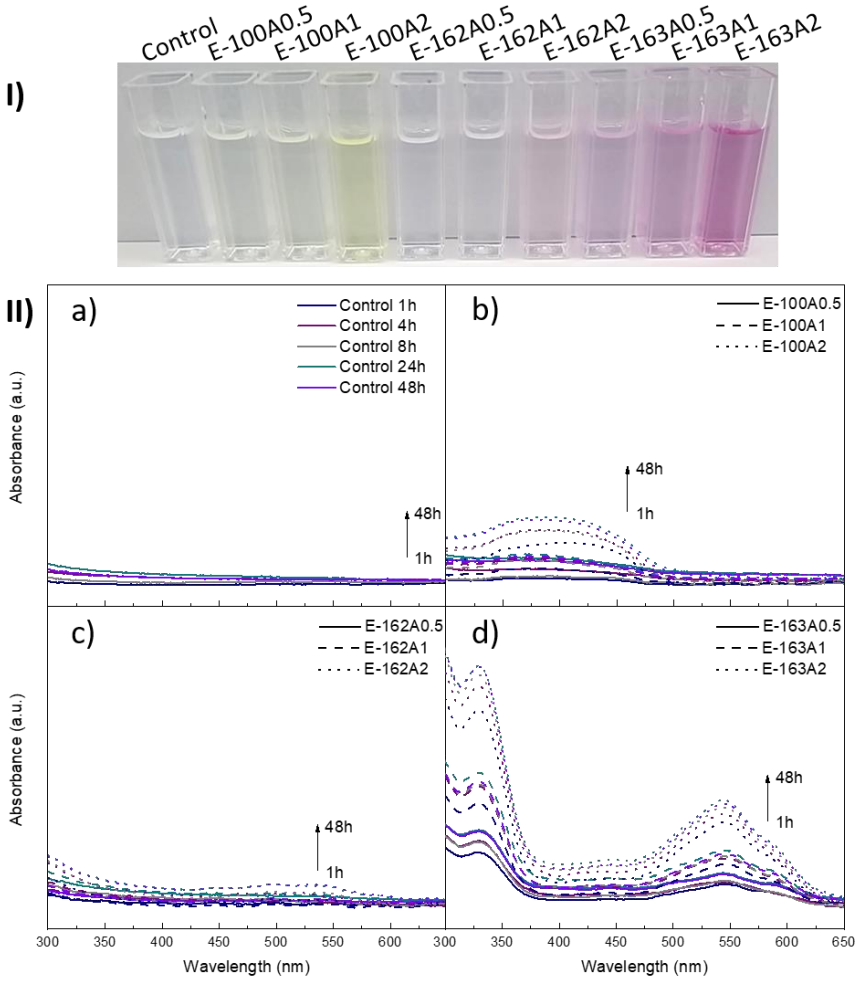

Figure 4. I) 50EtOH food simulant after the immersion of gelatin films with different concentrations (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163) for 48 h. **II)** UV-Vis spectra for 50EtOH food simulant during the immersion of gelatin films with different concentrations (A0.5, A1 and A2) of colourants: a) Control, b) E-100, c) E-162 and d) E-163, as a function of time (1, 4, 8, 24 and 48 h).

The colour retention of the films was also evaluated by film characterization (**Figure 5 I-II)**. E-100 and E-163 films almost completely lost their colour during film immersion and so, their absorbance values notably decreased, and with lower values observed as the colourant concentration decreased. However, E-162 films almost completely maintained their initial colour, as shown in the absorbance spectra of the films (**Figure 5-II c**), which barely changed over time.

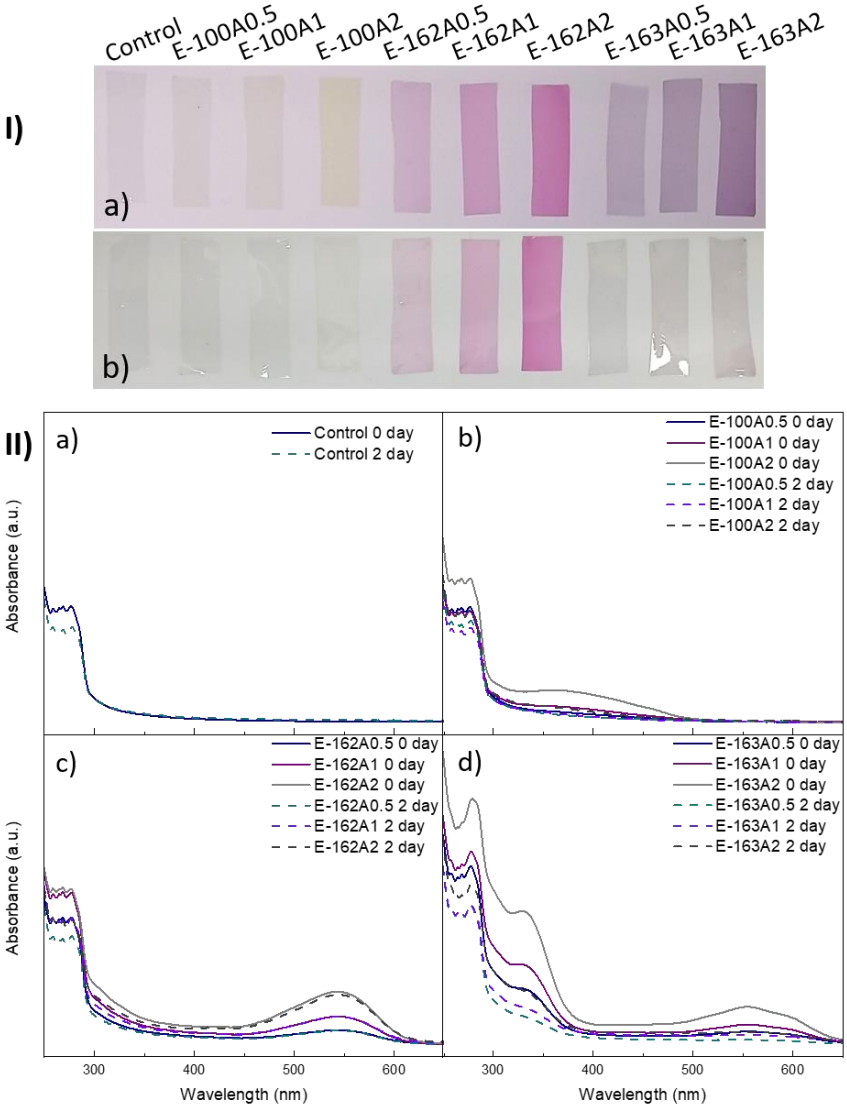

Figure 5. I) Gelatin films with different concentration (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163) a) before and b) after immersion in 50EtOH for 48 h. **II)**

UV-Vis spectra for gelatin films with different concentration (A0.5, A1 and A2) of colourants a) Control, b) E-100, c) E-162 and d) E-163, before and after immersion in 50EtOH for 48 h.

In order to better understand the retention capacity of the films, and the chemical affinity between compounds, protein-colourant interactions were studied by FTIR analysis, and the spectra are shown in **Figure 6**. In the analysis of individual components (**Figure 6 a**), the main absorption bands of gelatin were related to C=O stretching at 1630 cm^{-1} (amide I), N-H bending at 1530 cm^{-1} (amide II) and C-N stretching at 1230 cm^{-1} (amide III) (Frazier & Srubar, 2016). Regarding the colourants, E-100 presented similar infrared spectrum to pure polysorbate 80 (Krstonošić, Milanović & Dokić, 2019), suggesting the predominant presence of the emulsifier in E-100 colourant sample, which made it difficult to observe the characteristic IR frequencies of curcumin. However, some signals were present in the spectral range 1650-1500 cm^{-1} which could be related to the characteristic absorption bands of curcumin attributed to aromatic moiety C=O stretching (1625 cm^{-1}), C-C benzene ring stretching vibrations (1586 cm^{-1}) and C=C stretching vibration band (1513 cm^{-1}) (Roy & Rhim, 2020).

The FTIR spectrum of E-162 presented a broad and strong band centred at 3284 cm^{-1} related to -OH stretching, the asymmetric and symmetric stretching of -CH and $-CH_2$ at 2925 and 2880 cm^{-1}, stretching of C=O functional group at 1606 cm^{-1}, C-N stretching at 1120 cm^{-1} and a band at 1039 cm^{-1} associated to the asymmetric stretching of the glyosidic linkages (C-O-C) (Sengupta, Mondal & Mukherjee, 2015; Tran, Athanassiou, Basit & Bayer, 2017). As for E-163, the FTIR spectrum showed a broad band centred at 3290 cm^{-1} assigned to -OH stretching, the asymmetric and symmetric stretching of -CH and $-CH_2$ at 2926 and 2886 cm^{-1}, C=O and C=C stretching of aromatic rings at 1710 cm^{-1} and at 1596 cm^{-1}, respectively, pyran rings stretching at 1226 cm^{-1} and a band at 1040 cm^{-1} associated to the stretching of the glyosidic linkages (Pereira Jr. et al., 2015).

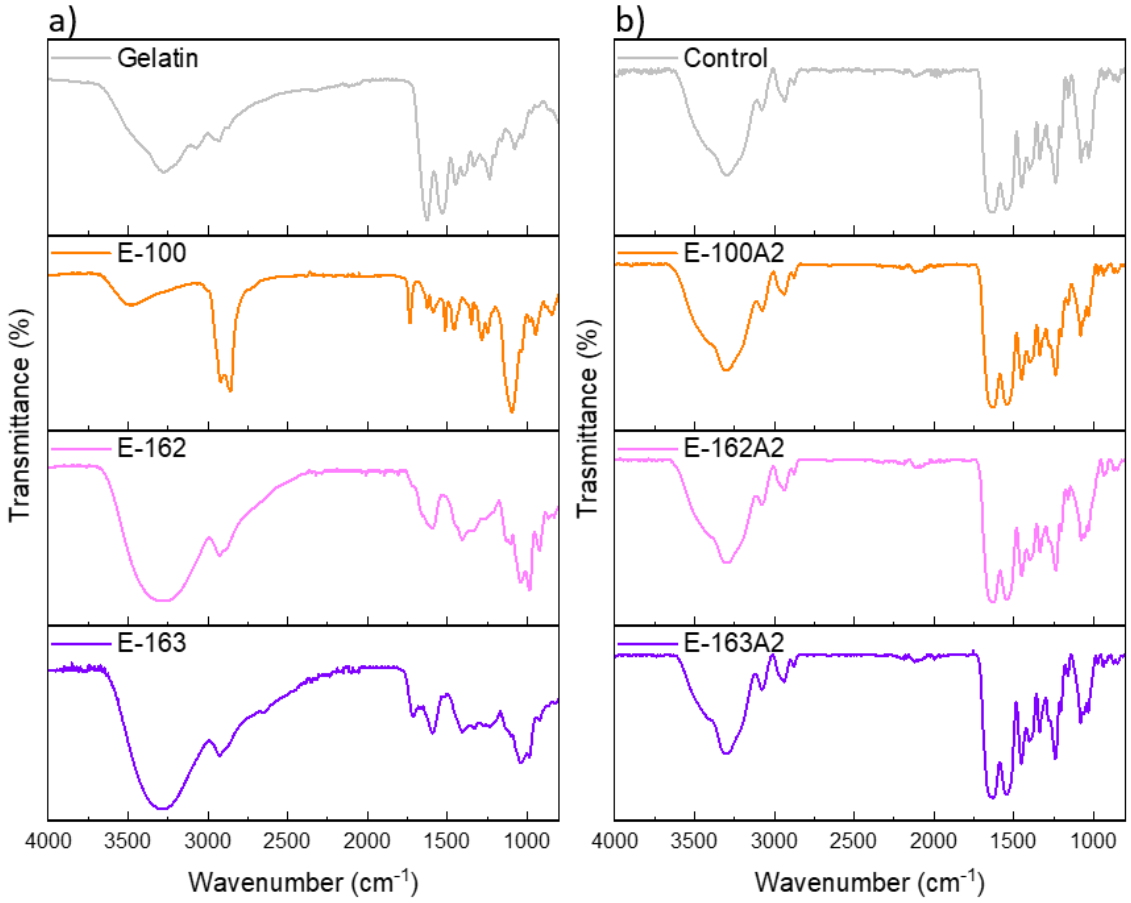

Figure 6. FTIR spectra of a) individual components used in film preparation and b) gelatin films as a function of added colourants (E-100, E-162 and E-163, absorbance (A) 2).

Films containing colourants (**Figure 6 b**) showed no significant changes in their spectra compared to control spectra. This could be related to low relative molecular masses of colourant molecules that could lead to relatively weak absorption bands as compared to those of the protein molecules. However, the absorption band centred at 1060 cm^{-1}, associated with the hydroxyl group, presented some intensity variations which could indicate the formation of intermolecular hydrogen bonds, as well as weak interactions of colourants with gelatin such as physical interactions (Deng, Kang, Liu, Feng, & Zhang, 2017; Yang et al., 2018; Roy & Rhim, 2020).

In order to further analyse the retention behaviours of films containing colourants, the gelatin structure and film morphology were analysed using XRD, mechanical testing and SEM.

Regarding the XRD results (**Figure 7**), all patterns displayed two diffraction peaks: a sharp peak at 7.4°, corresponding to the residual triple-helix from native collagen, and a broad peak at 21°, related to the crystallinity of the gelatin (Liu, Antoniou, Li, Ma, & Zhong, 2015). The addition of colourants had little effect on the rearrangement of the triple-helix and the reordering of the gelatin crystalline region, even at the highest colorant concentration, since only a small lowering in peak intensities was observed. These changes can be related to the small percentage of colourants in films that could slightly affect the intermolecular interactions.

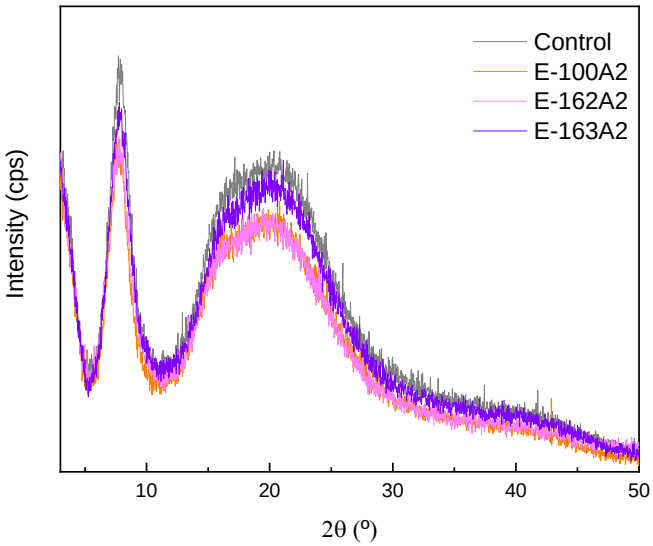

Figure 7. XRD patterns of the gelatin films as a function of added colourants (E-100, E-162 and E-163, initial absorbance (A) 2).

As the triple-helix content of gelatin can be related to its mechanical properties (Bigi, Panzavolta, & Rubini, 2004), the mechanical properties of the films were analysed. The small variations of both triple-helix content and the crystallinity of the gelatin films did not produce significant changes in the mechanical properties of the films. This is shown by the tensile strength (TS) and elongation at break (EB) values (**Table 3**) not showing a considerable improvement when different amounts of colourants were added. These results indicate that the

gelatin structure barely changed with the addition of colorants, irrespective of colourant type and concentration.

Table 3. Tensile strength (TS) and elongation at break (EB) of gelatin films with different concentrations (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163). Two means followed by the same number/letter in the same row are not significantly (P > 0.05) different through the Tukey's multiple range test. (n=5)

Films	TS (MPa)	EB (%)
Control	$59.21 \pm 5.35^{1/a/A}$	$2.84 \pm 0.32^{1/a/AB}$
E-100A0.5	64.78 ± 4.28^{1}	3.18 ± 0.46^{1}
E-100A1	68.44 ± 6.00^{1}	3.04 ± 0.48^{1}
E-100A2	63.75 ± 4.93^{1}	2.86 ± 0.51^{1}
E-162A0.5	62.55 ± 6.92^{a}	2.39 ± 0.30^{a}
E-162A1	66.05 ± 5.92^{a}	2.74 ± 0.35^{a}
E-162A2	66.28 ± 3.43^{a}	2.62 ± 0.41^{a}
E-163A0.5	71.65 ± 4.98^{B}	3.22 ± 0.52^{A}
E-163A1	69.07 ± 5.21^{B}	2.65 ± 0.35^{AB}
E-163A2	66.43 ± 4.73^{AB}	2.37 ± 0.15^{B}

As for SEM, images of the cross-section (CS) of films are shown in **Figure 8**. The continuous and uniform structure of the control films was not affected by the addition of colourants, event at the highest colourant concentrations. This indicates that gelatin and the colourants were homogeneously mixed and were compatible with each other (Liu et al., 2019).

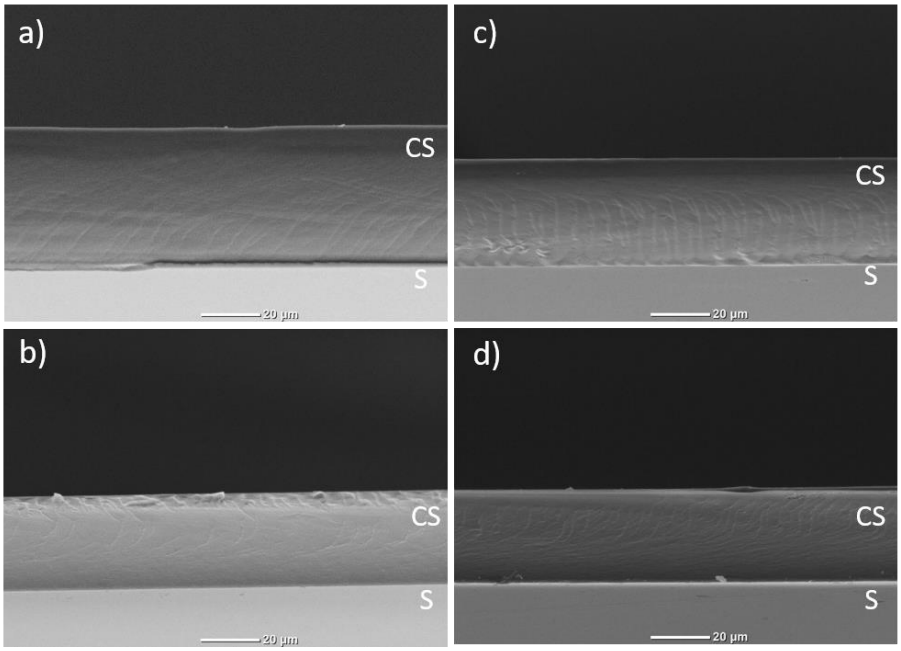

Figure 8. SEM cross-sectional micrographs of films a) control, b) E-100, c) E-162 and d) E-163 (initial colourant absorbance 1). CS (cross-section) and S (surface) of the films. Slight differences in thickness are related to small variation in film inclination grades, which led to different apparent thicknesses.

3.3 Colour response analysis of the films

The addition of colourants into the gelatin formulation provided films with the capacity of sensing and exhibiting pH alterations (a communication function) (Musso, Salgado, & Mauri 2017; Qin Liu, Zhang, & Liu, 2020), since the films containing food colorants presented quick colour changes when the pH was varied (**Figure 9 a-c**). These colour variations were clearer as the colourant concentration increased, especially in the E-100 films, and the colour responses to pH variations were related to molecular structural transformations of the main colouring compounds (curcumin, betanin and anthocyanins), which were dependent on the pH of the environment (Kennedy & Waterhouse, 2000; Zumdahl & DeCoste, 2013). However, some colour fading was observed after pH variations and during film drying, which were related to a destabilization of the main colouring compounds with the pH, especially under basic conditions (pH ≥ 10).

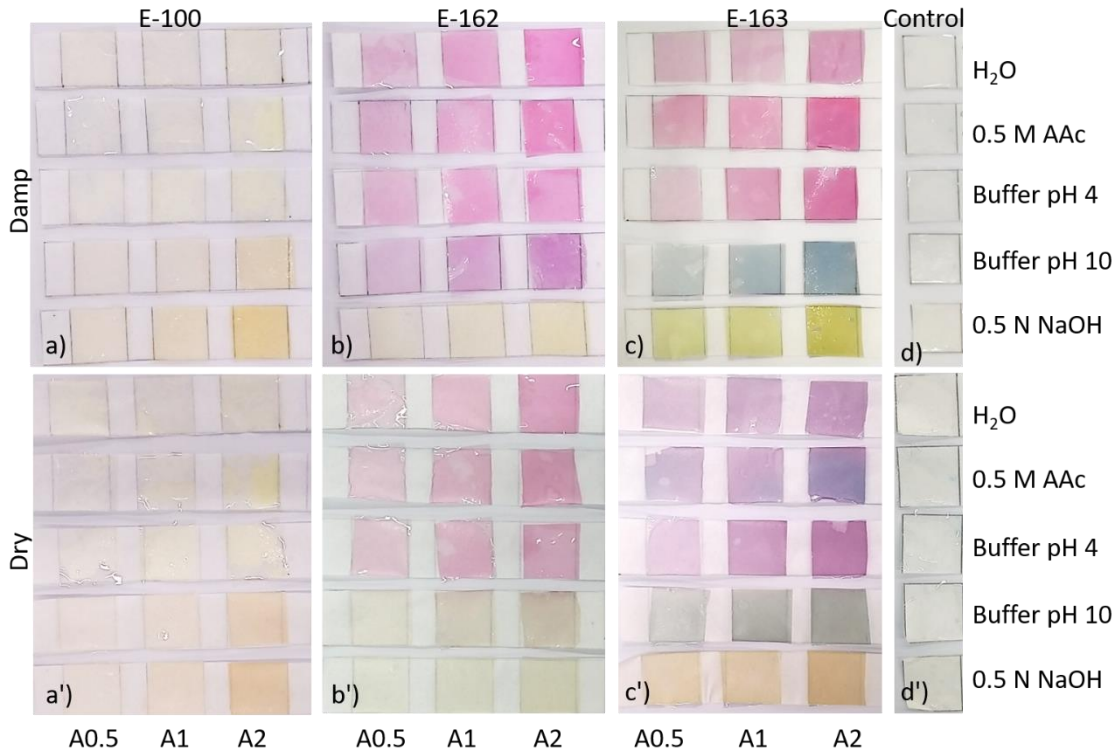

Figure 9. Colour change capacity of gelatin films containing different amounts (initial absorbance (A) 0.5, 1 and 2) of colourants (E-100, E-162 and E-163) as a function of pH.

Films exposure to pH ≥10 resulted in colour changes from yellow to reddish-yellow in the E-100 films (**Figure 9 a**), with a more intense colour under more alkaline conditions. These colours were more noticeable when the films were dried (**Figure 9 a'**), indicting a good stability of the main colouring compound (curcumin). Regarding the E-162 films (**Figure 9 b**), colour changes from reddish-purple to violet were seen when the films were exposed to pH 10. A higher alkalinisation (pH ≈14) turned the red-purple films light yellow, which points to colour degradation (Khan, 2016). The colour of the samples modified by the pH 10 buffer showed light yellow colours after drying (**Figure 9 b'**), also indicating betanin degradation. When the pH was modified in the E-163 films (**Figure 9 c**), the colour changed from mauve to both reddish and blue, when the films were exposure to pH values of 3, 4, & 7, and pH 10, respectively, and clearer colour change response was observed in higher acid and basic environments than at neutral pH values. In addition, light green colours were also seen under stronger alkaline conditions (0.5 M

NaOH). Once the films were dry (**Figure 9 c'**), the colour of the samples modified by water, 0.5 M AAC and the buffer pH 4, turned to violet colour, while films modified by pH 10 buffer showed a green-grey colour. Films damped with 0.5 M NaOH lost their colour once they were dry, due to destabilization of the anthocyanin molecules (Kennedy & Waterhouse, 2000).

Considering the intended use of these films for intelligent food packaging, their release from films into food could occur, as shown in the colour retention study, which might affect the colour response capacity of the films. Therefore, the colour response of the films after immersion in 50EtOH at 4 °C for 2 days was analysed. As all of the colourants exhibited colour change in the pH 10 buffer, and films with an initial absorbance value of 2 maintained better the colour after immersion in 50EtOH, the ability of E-100A2, E-162A2 and E-163A2 films to change the colour at pH 10 environments was analysed (**Figure 9**). As can be seen, the films presented similar behaviour as with the non-immersed films (**Figure 8)**, since they maintained their capacity to change colour after immersion. Again yellow, violet and blue colours were observed after the pH modification. All of the colourant containing films presented a clear colour change in a basic environment. This showed the suitability of the films to monitor the freshness and quality of seafood products, since volatile basic nitrogen compounds such as ammonia, are produced during spoilage (Ma, Ren, Gu, & Wang, 2017).

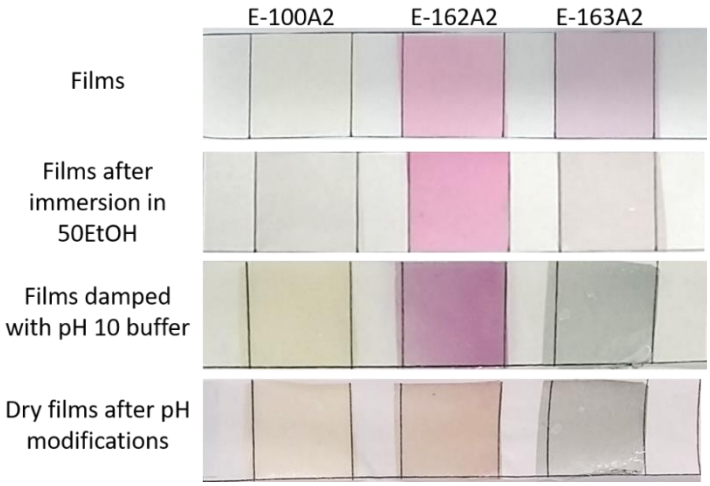

Figure 9. pH modification with pH 10 buffer of E-100, E-162 and E-163 films (initial colourant absorbance value 2) after immersion in 50EtoH food simulant for 48 h.

4. Conclusions

The gelatin films prepared in this work were homogeneous, transparent and showed high resistance against UV light, which are desirable properties for food packaging applications. The addition of different concentrations of colourants affected the colour and wettability properties of films, which made the films yellower and more hydrophilic, and redder and less hydrophilic when E-100 and E-162/E-163 colourants were added, respectively. The solubility and colour retentions tests showed low solubility values for the films in a fatty food simulant. Along with gelatin, some colourants (E-100 and E-163) were released from the films, while the E-162 films showed excellent colour retention, irrespective of the colourant concentration. Furthermore, the addition of colourants into the gelatin formulation provided films with the capacity of sensing and exhibiting pH alterations, even after immersion of films in a 50EtOH food simulant. An increase of colourant concentration provided a clearer colour response to pH variations, especially in E-100 films, and films containing the E-163 colourant showed superior colour variations to a wider range of pHs. The communication function of the colourant containing films could be used for sensing the freshness and quality of foodstuffs such as seafood products. Further research is required to analyse the possible interaction between gelatin and the colourants, especially for E-162.

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