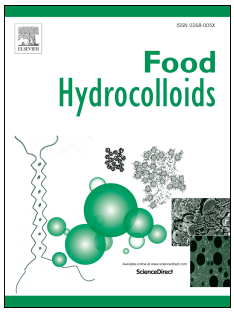
Accepted Manuscript



Smart edible films based on gelatin and curcumin

Yanina S. Musso, Pablo R. Salgado, Adriana N. Mauri

PII: S0268-005X(16)30761-5

DOI: 10.1016/j.foodhyd.2016.11.007

Reference: FOOHYD 3668

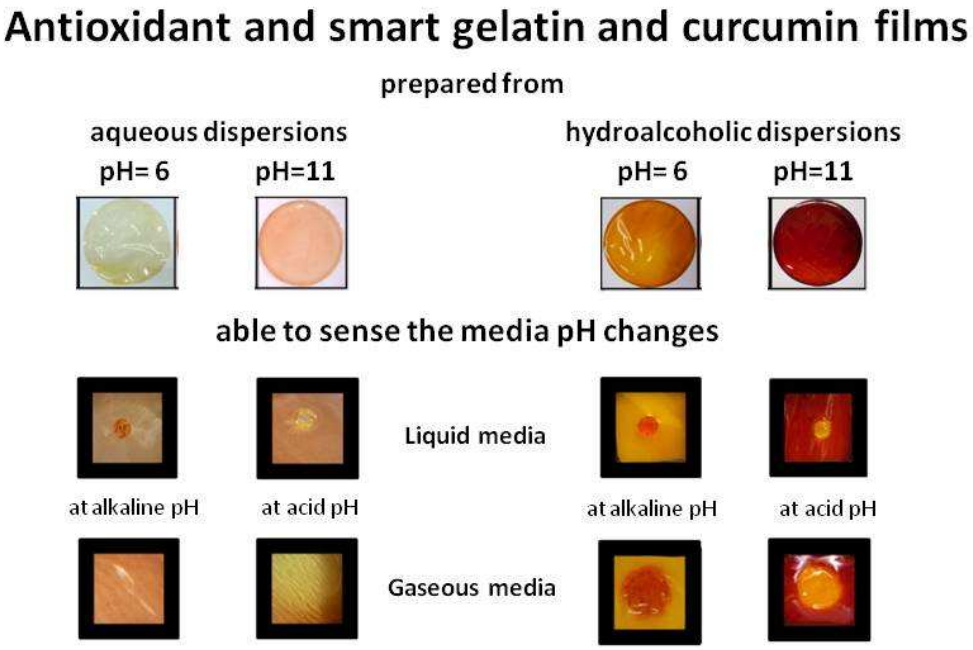
To appear in: *Food Hydrocolloids*

Received Date: 10 August 2016

Revised Date: 4 November 2016

Accepted Date: 7 November 2016

Please cite this article as: Musso, Y.S., Salgado, P.R., Mauri, A.N., Smart edible films based on gelatin and curcumin, *Food Hydrocolloids* (2016), doi: 10.1016/j.foodhyd.2016.11.007.


Antioxidant and smart gelatin and curcumin films
prepared from
aqueous dispersions
pH= 6
pH=11
hydroalcoholic dispersions
pH= 6
pH=11
able to sense the media pH changes
Liquid media
at alkaline pH
at acid pH
at alkaline pH
at acid pH
Gaseous media

Smart edible films based on gelatin and curcumin

Yanina S. Musso, Pablo R. Salgado and Adriana N. Mauri*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET CCT La Plata y Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 116 S/N°, (B1900JJ) La Plata, República Argentina.

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, (C1033AAJ) Ciudad de Buenos Aires, República Argentina.

*Author to whom correspondence should be addressed: Tel.: +54-221-4249287; fax: +54-221-4254853. E-mail: anmauri@quimica.unlp.edu.ar

Abstract

This work studied the preparation of edible smart films based on gelatin and curcumin. Films were prepared by casting using water and an ethanol-water mixture as solvents. The addition of curcumin, besides affecting the physicochemical properties of gelatin films, colored them depending on the pH of the film-forming dispersion (yellow at pH=6 and red at pH=11). It also provided films with important antioxidant properties, but no antimicrobial activity. The response of these materials against pH changes was evaluated simulating their contact with liquid and semisolid foods, and with a container headspace at acid and alkaline pH. In all tests, gelatin films with curcumin added could modify their color after being in contact with media of different pH. The use of an ethanol-water mixture as solvent was a good alternative to intensify film color and the visualization of their response capacity against pH changes, as well as to increase the antioxidant properties and hydrophobicity of films. These edible films could be used as smart food packaging, since they could inform consumers if the product was suitable for consumption through their capacity to sense pH changes.

Keywords: smart packaging, curcumin, protein film, pH indicators, gelatin, food spoilage sensor.

1. Introduction

Changes in consumer preferences have led to innovations and developments in new packaging technologies. 'Smart packaging' is a broad term encompassing a range of relatively new packaging concepts, most of which can be placed in one of the two main categories: active packaging and intelligent packaging (Kerry, 2012). Active packaging has been defined as one that changes the condition of packed food to extend shelf-life or to improve safety or sensory properties maintaining its quality, whereas intelligent ones refer to those which monitor the condition of packaged foods to give information about their quality to manufacturers, retailers or consumers (Ahvenainen, 2003). These last materials often attempt to sense environmental changes or specific compounds generated during food packaging or storage, in order to inform the freshness or microbiological quality of food (Biji, Ravishankar, Mohan, & Srinivasa Gopal, 2015). Media pH could be modified during food storage, through changes in the concentrations of organic acids (such as *n*-butyrate, *L*-lactic acid, *D*-lactate and acetic acid), and volatile compounds development (such as trimethylamine, dimethylamine, histamine, hypoxanthine, putrescine, tyramine, cadaverine, and hydrogen sulphide, among others) as a result of microorganisms growth and metabolism (Al Bulushi, Poole, Deeth, & Dykes, 2009; Ruiz-Capillas & Jiménez-Colmenero, 2005). Thus, pH changes could be considered as potential indicators of food spoilage.

In previous work (Musso, Salgado, & Mauri, 2016), gelatin-based films capable of sensing changes in the surrounding pH medium were developed by adding known acid-base indicators methyl orange, neutral red and bromocresol green to the formulation. These indicators were used as model systems to test if proteins that can act as buffer systems, due to their chain ionizable side groups, allowed films to change their color

when in contact with gaseous, liquid and semisolid media with a different pH. Evidence showing that the protein matrix did not interfere with the possible discoloration of the acid-base indicators and the fact that films could change their color according to media pH, pushed to find food grade dyes that could replace these synthetic indicators in order to develop real food packaging materials capable of sensing pH changes.

Curcumin is the product obtained by solvent extraction from turmeric –the ground rhizomes of *Curcuma longa L.* – and later purification by crystallization. It is widely used as a spice and coloring agent in food by virtue of its yellowish-orange color and pleasant aroma (Martins, Roriz, Morales, Barros, & Ferreira, 2016). It has also been used in a variety of pharmaceutical applications, because it exhibited many interesting biological activities such as antiviral, anti-inflammatory, antimicrobial, antioxidant, anti-HIV, anti-Parkinson, anti-Alzheimer's, anti-angiogenesis, free radical scavenging activity, and anticancer (Pulido-Moran, Moreno-Fernandez, Ramirez-Tortosa, & Ramirez-Tortosa, 2016). The IUPAC name of curcumin is (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6hepadiene-3,5-dione. In solution, it exhibits a keto-enol tautomerism and, depending on the solvent, up to 95% could be in the enol form. In the pH range 1-7, the majority of diferuloyl methane species are in the neutral form, water solubility is very low and solutions are yellow. At pH >8.5, solutions changed their color to red and their water solubility barely increased. However, due to its chemical structure, curcumin is highly soluble in ethanol, chloroform, dimethyl sulfoxide and oils (Mehanny, Hathout, Geneidi, & Mansour, 2016; Priyadarsini, 2014).

Curcumin was added in natural and synthetic polymer films in order to provide them with antioxidant and antimicrobial properties (Bajpai *et al.*, 2015; Govindaraj, Kandasubramanian, & Kodam, 2014; Liu *et al.*, 2016; Mayet *et al.*, 2014). Maniglia,

Domingos, de Paula, & Tapia-Blácido (2014 and 2015) used turmeric dye extraction residue for the production of bioactive films. As far as we know, only Kuswandi, Jayus, Larasati, Abdullah, & Heng (2012) used curcumin to develop a sensor for the detection of volatile amines. They did it by the absorption method of curcumin onto bacterial cellulose membrane (also called *nata de coco*), and used it as a sticker sensor for real-time monitoring of shrimp spoilage, as this membrane was highly sensitive toward acid–base reactions.

The aim of this work was to prepare active gelatin films capable of sensing pH changes by the addition of curcumin to films formulations. Taking into account the hydrophobicity of curcumin and the fact that films were obtained by a casting technique, water and an ethanol-water mixture were analyzed as solvents for film-forming dispersions.

2. Material and Methods

2.1 Materials

Bovine gelatin with 240 Bloom (Kraft Foods, Argentina) was used as protein source. Its protein content, as measured by the Kjeldahl method (AOAC, 1995), was 87.8±0.6% (w/w, dry weight; N×5.5). Glycerol (Anedra, Argentina) was used as film plasticizer. Curcumin (Chr Hansen, Argentina) was used as colorant. All the other reagents used in this study were of analytical grade.

2.2 Films preparation

Films were prepared by casting using water and an ethanol-water mixture (1:1 v/v) as solvents. In each case, two dispersions were prepared separately: one containing 10% w/v of gelatin in water at 100 °C and another containing 0.04% w/v of curcumin in water or ethanol respectively, at ambient temperature. Equal volumes of protein and curcumin dispersions were mixed with magnetic stirring, adding glycerol (1.25% w/v) as plasticizer and the pH of each dispersion was adjusted to 6 and 11 with 2 mol/L HCl and 2 mol/L NaOH respectively. Finally, 10 mL of each film-forming dispersion were cast onto polystyrene Petri dishes (64 cm^2) and dried in an oven with air flow circulation (Yamato, DKN600, USA) at 60 ºC for 3 h. The resulting films were preconditioned during 48 h at 20 ºC and 58% relative humidity (in desiccators with saturated solutions of NaBr) just before being peeled from the casting surface and characterized. Furthermore, control gelatin films without the incorporation of curcumin in both solvents at pH= 6 and 11, were prepared as described previously. **Table 1** summarizes film nomenclature and the final formulation of film-forming dispersions. Three independent batches for each type of protein film were performed.

2.3 Films characterization

Thickness: Film thickness was measured by a digital coating thickness gauge (Check Line DCN-900, USA). Measurements were done at five positions along the rectangular strips for the tensile test, and at the center and at eight positions round the perimeter for the water vapor permeability (WVP) determinations. The mechanical properties and WVP were calculated using the average thickness for each film replicate.

Color: Film color was determined with a Konica Minolta Chroma Meter CR-400 (Konica Minolta Chroma Co., Osaka, Japan) set to C illuminant/2° observer. A CIE-Lab

color scale was used to measure the degree of lightness (L^*), redness ($+a^*$) or greenness ($-a^*$), and yellowness ($+b^*$) or blueness ($-b^*$) of the films. The instrument was calibrated using a white standard plate with color coordinates of $L^*_{standard}$ = 97.55, $a^*_{standard}$ = -0.03 and $b^*_{standard}$ = 1.73 provided by Minolta. Films color was measured on the surface of this standard plate and total color difference (ΔE^*) was calculated as follow:

$$\Delta E^* = [(L^*_{film} - L^*_{standard})^2 + (a^*_{film} - a^*_{standard})^2 + (b^*_{film} - b^*_{standard})^2]^{0.5} \quad (1)$$

Values were expressed as the means of nine measurements on different areas of each film.

UV-Visible absorption spectra: Each film specimen was cut into a rectangular piece and placed directly in a spectrophotometer test cell. A spectrum (from 200 to 800 nm) of each film was obtained in an UV-Vis spectrophotometer (Biotek, synergy HT, USA). Measurements were performed using air as reference. All determinations were performed in triplicate.

Moisture content (MC): Small specimens of films were collected after conditioning, cut and weighed before and after oven drying at 105°C for 24 h, ASTM D644-99, (ASTM 2004). MC values were determined in triplicate for each film, and calculated as the percentage of weight loss relative to the original weight.

Water solubility (WS): WS was determined as was described by Gontard, Duchez, Cuq, & Guilbert (1994) with slight modifications. Three pieces of films were weighed (diameter = 2 cm; ~0.03-0.05 g) and immersed in 50 mL of distilled water. The system was sealed, shaken at 100 rpm for 24 h at 20°C (Ferca, TT400 model, Argentina), and then filtered through Whatman n°1 filter paper (previously dried and weighed) to

recover the remaining undissolved film, which was desiccated at 105°C for 24 h. WS was calculated as follows:

$$WS = [(P_0 \cdot (100 - MC)) - P_f] \cdot 100 / [P_0 \cdot (100 - MC)] \quad (2)$$

Where P_0 = initial film weight (g), P_f = final dry film weight (g), MC = moisture content (%). All tests were carried out in triplicate.

Water vapor permeability (WVP): Water vapor permeability tests were conducted according to ASTM method E96-00 (ASTM, 2004) with some modifications. Each film sample was sealed over a circular opening of 0.00185 m^2 in a permeation cell that was stored at 20°C in desiccators. To maintain a 75% relative humidity (RH) gradient across the film, anhydrous silica (0% RH_c) was placed inside the cell and a saturated NaCl solution (75% RH_d) was used in the desiccators. The RH inside the cell was always lower than outside, and water vapor transport was determined from the weight gain of the permeation cell. When steady-state conditions were reached (about 1 h), eight weight measurements were made over 5 h. Changes in the weight of the cell were recorded and plotted as a function of time. The slope of each curve ($\Delta m/\Delta t$, g H_2O s^{-1}) was obtained by linear regression and the water vapor transmission rate (WVTR) was calculated from the slope divided by the permeation cell area (A, in m^2). WVP (g H_2O Pa^{-1} s^{-1} m^{-1}) was calculated as:

$$WVP = [WVTR / (P_V^{H2O} \cdot (RH_d - RH_c))] \cdot d \quad (3)$$

Where: WVTR = water vapor transmission rate (g H_2O s^{-1} m^{-2}), P_V^{H2O} = saturation water vapor pressure at test temperature (2339.27 Pa at 20 °C), RH_d - RH_c = relative humidity gradient across the film -expressed as a fraction- (0.75), A = permeation area (m^2), and d = film thickness (m). Each WVP value represents the mean value of three samples taken from different films.

175 *Mechanical properties:* Tensile strength (TS), Young´s modulus (YM) and elongation
176 at break (EAB) of films were determined following the procedures outlined in the
177 ASTM method D882-02 (ASTM, 2004), using a texture analyzer TA.XT2i (Stable
178 Micro Systems, Surrey, England) equipped with a tension grip system A/TG. Films
179 probes of 90 mm length and 6 mm width were used. The initial grip separation was set
180 at 50 mm and the crosshead speed at 0.4 mm s^{-1}. Measurements were made at 20°C in a
181 temperature-controlled room. The curves of force (N) as a function of distance (mm)
182 were recorded by the Texture Expert V.1.15 software (Stable Micro Systems, Surrey,
183 England). Tensile properties were calculated from the plot of stress (tensile force/initial
184 cross-sectional area) versus strain (extension as a percentile of the original length). TS
185 and EAB were determined directly from the stresses-train curves, and YM was
186 determined as the slope of the initial linear portion of this curve. Reported values are the
187 average of at least twelve replications taken from different films for each formulation.
188
189 **2.4 Antioxidant and antimicrobial properties of films**
190 *Antioxidant capacity of films:* The supernatants obtained in the WS test were used for
191 testing the film antioxidant capacity based on two different antioxidant mechanisms: the
192 radical scavenging capacity and the reducing capacity.
193 The radical scavenging capacity was measured using $ABTS^{\bullet+}$ (2,2´-azino-bis-(3-
194 ethylbenzothiazoline-6-sulfonic acid) radical cation decoloration assay according to
195 Salgado, López-Caballero, Gómez-Guillén, Mauri, & Montero (2012). Samples were
196 mixed with ABTS reagent. The mixture was then left to stand at 30 °C for 10 min and
197 absorbance values were read at 734 nm (Biotek, synergy HT, USA). Results were
198 expressed as concentration of ascorbic acid equivalent per g of protein film based on a

199 standard curve of ascorbic acid, which relates the concentration to the absorbance at 734
200 nm. Determinations were carried out in triplicate.
201 The reducing capacity was measured following the ferric ion reducing capacity (FRAP)
202 assay, accoding to Salgado *et al.* (2012). Samples were incubated (at 37ºC) with
203 distilled water and FRAP reagent (containing 2,4,6-tripyridyl-s-triazine and
204 $FeCl_3.7H_2O$) in sodium acetate buffer pH 3.6. Absorbance values were read at 595 nm
205 after 30 min (Biotek, synergy HT, USA). Results were expressed as mmol $FeSO_4.7H_2O$
206 equivalents per g of protein film based on a standard curve of $FeSO_4.7H_2O$, which
207 relates the concentration to the absorbance at 595 nm. All determinations were carried
208 out in triplicate.
209 *Antimicrobial properties of films:* The "zone of inhibition" assay on solid media was
210 used for determination of the antimicrobial effects of gelatin films against *Salmonella*
211 *enteritidis*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* (Salgado,
212 López-Caballero, Gómez-Guillén, Mauri, & Montero, 2013). Gelatin films were
213 aseptically cut into 10 mm diameter discs and then placed on solid nutrient agar (Biokar
214 diagnostics) plates, which had been previously spread with 10 µL of inoculum
215 containing 10^8 CFU/mL of tested bacterium. Plates were incubated at 37 °C for 48 h.
216 The plates were examined visually for "zone of inhibition" of the film discs, and the
217 diameter of the zone was measured with a gauge. Tests were done in duplicate.
218
219 **2.5 Films response to pH changes**
220 Each film was faced with liquid, semisolid and gaseous media of different pH: i) adding
221 a drop of 2 mol/L HCl or 2 mol/L NaOH directly on films; ii) placing the films in
222 contact with gels prepared from gelatin solutions at 7.5% w/v at pH= 2.5, and 11; and
223 iii) exposing the films to gaseous atmospheres generated by acetic acid glacial ($C_2H_4O_2$,

pK$_a$~4.8, Anedra, Argentina) and ammonia (NH_3, pK$_a$~9.3, Anedra, Argentina) (Musso *et al.*, 2016). Photographs of films before and after 30 min contacting it with those media of different pH were taken with a digital camera (Kodak M853, USA) and color variations were measured using a colorimeter (Konica Minolta Chroma Meter CR-400), as described above.

2.6 Statistical analysis

Results were expressed as mean ± standard deviation and were analyzed by analysis of variance (ANOVA). Means were tested with the Tukey's HSD (Honestly Significant Difference) test for paired comparison, with a significance level $\alpha = 0.05$, using the Statgraphics Plus version 5.1 software (Statgraphics, USA).

3. Results and Discussion

3.1 Appearance and optical properties of films

Gelatin films prepared with or without curcumin from aqueous or hydroalcoholic dispersions at pH 6 or 11 were homogeneous, thin and flexible. **Figure 1** shows their visual appearance. Gelatin films were colorless regardless of pH and the solvent used, while those with curcumin added became yellow and orange-red, depending on whether the dispersion pH was 6 or 11. These colorations were more intense for films prepared using the ethanol-water mixture as a solvent.

Color parameters *L**, *a**, *b** and *ΔE** of the studied films are shown in **Table 2**. Gelatin films of different pH did not show significant differences in color parameters –with the exception of those prepared with the ethanol-water mixture at pH 6 which present a higher *b** value–, being those obtained with ethanol-water mixtures rather clearer (higher *L**) than those obtained with only water as a solvent ($p<0.05$). Gelatin films

prepared with curcumin showed a significant increase ($p<0.05$) in a^*, b^* and ΔE^* values and a significant decrease ($p<0.05$) in L^*. This tendency was more marked for the films prepared using the ethanol-water mixture as solvent.

A more intense coloration in hydroalcoholic films can be due to curcumin's higher solubility in this medium than in water because of its hydrophobic nature (Priyadarsini, 2014). Supplementary Figure 1 shows the appearance of curcumin dispersions in both solvents. The development of color in these systems might imply that water can disperse it (panel A), whereas the hydroalcoholic mixture seems to dissolve it completely (panel B). The addition of gelatin and glycerol to the system (Supplementary Figure 1, panels C and D) apparently improves colorant dispersion and even modify its tonality, especially in aqueous dispersions.

Figure 2 shows the UV-visible absorption spectra of studied films. Those prepared only with gelatin (**Gw6**, **Gw11**, **Gew6**, and **Gew11**) showed the same spectra regardless of the pH and solvent used in film-forming solutions. They showed two absorption peaks at 205 nm and 230 nm attributed to peptide bonds, another at 260-280 nm corresponding to the aromatic amino acids absorption, and a minimum absorbance in the complete visible spectra range. Priyadarsini (2014) reported that the absorption spectrum of curcumin has two strong absorption bands, one in the UV region with maximum at 265 nm and another one in the visible region with a maximum ranging from 410 to 430 nm. The addition of curcumin into gelatin films increased the absorption peaks in the UV region, especially the one at 260-265 nm. Films at pH 11 (**GCw11** and **GCew11**) showed two new absorption peaks in the UV region with maximums at 345 nm and 395 nm, which could be attributed to curcumin degradation products under alkaline conditions, like *trans*-6(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic aldehyde, ferulic acid, feruloylmethane and vanillin (Wang *et*

al., 1997). Moreover, in the visible range of the spectra, yellow gelatin-curcumin films at pH 6 (**GCw6** and **GCew6**) had an absorption peak at 420 nm, while those at pH 11 (**GCw11** and **GCew11**) showed it at 460 nm according to their red coloring. These results also agree with those of Priyadarsini (2014), who reported that the absorption peak of curcumin was at 420 nm at pH<7.5 and at 467 nm at pH>10. It is worth noting that the gelatin-curcumin films prepared from hydroalcoholic dispersions (**GCew6** and **GCew11**) presented higher absorbance in the entire UV-visible range than those prepared from aqueous dispersions at the same pH (**GCw6** and **GCw11**) due to the better solubilization of curcumin.

3.2 Film response to pH changes

Figure 3 shows the response of all gelatin–curcumin films (**GCw6**, **GCw11**, **GCew6**, and **GCew11**) when in contact with acid and alkali liquids, semisolids and gases. All films showed the ability to sense pH changes, simulating that these changes could occur in a liquid or semisolid food, or in the headspace of a food container as the result of the reaction products of food spoilage. Yellow films at pH 6 (**GCw6** and **GCew6**) turned to orange-red when in contact with alkaline gases, liquids and semisolids while orange-red films (**GCw11** and **GCew11**) at pH 11 turned yellow in contact with acid media. And as expected, those films whose pH was near the one of the media did not change their color. Changes were more noticeable in those films obtained from hydroalcoholic dispersions due to their more intense coloration. Film responses were immediate and marked with liquid and gases of different pH, but less evident and slower with a semisolid medium, especially with films prepared from aqueous dispersions. Slower turning kinetics against semisolid media could probably be attributed to limited diffusive processes (Musso *et al.*, 2016). Kuswandi *et al.* (2012) used a

299 curcumin/bacterial cellulose sensor to measure the pH increase produced by the basic
300 spoilage volatile amines gradually released in the shrimp package headspace, which
301 subsequently changed its color from yellow to orange, and then to reddish orange as
302 spoilage indication.
303
304 *3.3 Effects of pH, solvent and curcumin addition on the physicochemical properties of*
305 *films*
306 **Table 3** shows the thickness, moisture content, water solubility and water vapor
307 permeability of the studied films. Gelatin films prepared from aqueous dispersions were
308 thinner than those prepared using the ethanol-water mixture as a solvent ($p<0.05$).
309 Ethanol can denature proteins by disrupting the side-chain intramolecular hydrogen
310 bonding, and allowing the formation of new hydrogen bonds between alcohol molecules
311 and protein side-chains. Salgado *et al*. (*in press*) showed how the conformation of
312 proteins in the film-forming dispersions affects the physicochemical properties of films.
313 It is evident that gelatin dissolved in ethanol-water mixtures produced films with a
314 lower degree of compaction, suggesting different chain molecular unfolding or cross-
315 linking within the protein network of the film (Denavi, Pérez-Mateos, Añón, Montero,
316 Mauri & Gómez-Guillén, 2009). These films showed lower moisture content and WVP
317 ($p<0.05$) and similar water solubility ($p>0.05$) than those prepared from aqueous
318 dispersions, regardless of the film-forming dispersion pH. The addition of curcumin
319 significantly increased ($p<0.05$) the thickness values of films prepared at pH 11 in
320 water and decreased ($p<0.05$) those of the films prepared at pH 6 in ethanol-water.
321 Curcumin also caused a decrease in the MC and WVP of films prepared from aqueous
322 dispersions ($p<0.05$) and only an increase in the MC of those prepared from
323 hydroalcoholic dispersions ($p<0.05$). It seems that both ethanol and curcumin increased

the hydrophobic character of gelatin films, without affecting WS ($p>0.05$). In all cases, the pH of film-forming dispersions (both aqueous and hydroalcoholic) did not modify ($p>0.05$) moisture content and water solubility of studied gelatin films with added curcumin or not. Only thickness and WVP were modified by changes in pH of aqueous film-forming dispersions: **GCw11** was thicker than **GCw6** ($p<0.05$), and **Gw11** had higher WVP than **Gw6** ($p<0.05$).

Table 4 shows the mechanical properties of films measured by tensile tests. Gelatin films obtained from aqueous dispersions showed mechanical properties similar to those published by other authors (Carvalho *et al.*, 2008; Nur Hanani, Roos, & Kerry, 2012;). particularly those at pH 11 presented higher tensile strength and elongation at break but similar Young´s modulus ($p<0.05$) than those at pH 6, in concordance with previous results (Musso *et al.*, 2016). With the addition of curcumin, as well as replacing the aqueous solvent by a hydroalcoholic mixture, films presented a significant decrease in Young's modulus (at least 50%) ($p<0.05$). Curcumin also diminished the TS of alkaline films in both studied solvents but only the EAB of aqueous ones at pH 11 ($p<0.05$). Moreover, ethanol addition as solvent decreased TS of all films –except those with curcumin at pH 11–, but only the EAB of films without curcumin at pH 11 ($p<0.05$).

However, it seems that both main effects, the presence of curcumin and the use of an ethanol-water mixture as a solvent, affect the cross-linking of gelatin films although no difference was observed in their water solubility. It is evident that when protein matrix hydrophobicity increases, due to modifications in its structure caused by changes in solvents, or by the addition of a more hydrophobic component such as curcumin, protein cross-linking changes, resulting in a decrease in the Young´s modulus. But this change would not affect matrix stretching neither its water solubility significantly. The amino-acid composition of gelatin –low in sulfur-containing amino acids– explains this

behavior. In other protein matrixes, such as soybean, sunflower, amaranth and gluten (among others), solubility and mechanical properties are determined by the possibility of cross-linking through disulfide bonds (Condés, Añón, & Mauri, 2015; Mauri & Añón, 2006; Salgado; Fernández, Drago, & Mauri, 2011).

3.4 Antioxidant and antimicrobial properties of films

Figure 4 shows the antioxidant properties of studied gelatin films as assessed by different methods: ABTS (**A** and **C panels**) and FRAP (**B** and **D panels**). Gelatin films without curcumin addition exhibited a low antioxidant capacity in both methods tested, especially at pH 6. These results could be attributed in part to some gelatin amino acids that can act as electron donors, reacting with free radicals to give rise to more stable products in ABTS assay or reducing ferric ion in FRAP assay (Salgado *et al*., 2012). The addition of curcumin to formulations increased the antioxidant properties of resulting films significantly ($p<0.05$), being these increments more noticeable at alkaline pH than at pH 6 ($p<0.05$). On the other hand, gelatin films with curcumin added or not, prepared from hydroalcoholic dispersions, showed higher antioxidant properties than those prepared using water as a solvent ($p<0.05$). Differences in protein cross-linking, as well as changes in the hydrophilic-hydrophobic nature of the protein matrix, should modify the retention or release of active principles in both gelatin and curcumin, affecting the antioxidant properties of the resulting films. As the chemical structure of curcumin changes with the pH of film-forming dispersions, it could differentially interact with gelatin, inducing films with different antioxidant properties. These results suggest that developed gelatin films with curcumin incorporated, especially those obtained using ethanol-water mixtures as a solvent at alkaline pH, could be used as active films with an important antioxidant activity.

Despite the antimicrobial activity of curcumin against different fungal and bacterial strains and viruses reported in literature (Moghadamtousi, Kadir, Hassandarvish, Tajik, Abubakar, & Zandi, 2014), the gelatin-curcumin films showed no antimicrobial activity against *S. enteritidis*, *E. coli*, *B. cereus*, and *S. aureus* (data not shown). This result could be attributed, at least partly, to the low curcumin concentration employed in the studied films (0.02 % w/v in film-forming dispersions). Niamsa & Sittiwet (2009) reported that an aqueous extract of *C. longa* had a minimum inhibitory concentration value of 4 to 32 g/L against *S. aureus* and *E. coli*. On the other hand, Lawhavinit, Kongkathip, & Kongkathip (2010) reported that an alcoholic extract of turmeric had a minimum inhibitory concentration of 30 ppm against *S. aureus.* The lack of antimicrobial activity in this work may also be attributed to interactions between curcumin and gelatin. Salgado *et al.* (2012) reported that sunflower protein films containing phenolic compounds did not show antimicrobial properties due to the important interactions between phenolic compounds and proteins in films at alkaline pH.

4. Conclusions

It was possible to prepare smart gelatin films by adding curcumin to formulations. The resulting films showed antioxidant properties and were able to sense media pH by changing film color. Improvements in both behaviors were observed using ethanol-water mixtures instead of water as a solvent in film-forming dispersions. These films showed higher antioxidant activity and susceptibility to media pH. These edible materials could be used as smart food packaging as they could provide information about food spoilage indirectly through media pH measurement and extend the shelf-life of food through the material’s antioxidant properties.

Acknowledgements

The authors are thankful to the National Agency of Scientific and Technological Support (PICT-2010-1837, PICT-2013-2124) and the Universidad Nacional de La Plata (11/X618 and 11/X750) for their financial support.

References

Ahvenainen, R. (2003). Active and intelligent packaging: An introduction, in R. Ahvenainen (Ed.), *Novel food packaging techniques*, Cambridge: Woodhead, 5-21.

Al Bulushi, I., Poole, S., Deeth, H. C. & Dykes, G. A. (2009). Biogenic Amines in Fish: Roles in Intoxication, Spoilage, and Nitrosamine Formation—A Review. *Critical Reviews in Food Science and Nutrition*, 49 (4), 369-377.

AOAC. (1995). *Official methods of analysis of AOAC international*. (16th ed.), Horowitz, Washigton DC, USA.

ASTM. (2004). Annual book of ASTM standards. ASTM International: Philadelphia, PA, USA.

Bajpai, S. K., Chand, N., & Ahuja, S. (2015). Investigation of curcumin release from chitosan/cellulose microcrystals (CMC) antimicrobial films. *International Journal of Biological Macromolecules*, 79, 440–448.

Biji, K. B., Ravishankar, C. N., Mohan, C. O., & Srinivasa Gopal, T. K. (2015). Smart packaging systems for food applications: a review. *Journal of Food Science and Technology*, 52(10), 6125–6135.

421 Cao, N., Hu, Y., & He, J. (2007). Mechanical properties of gelatin films cross-linked,
422 respectively, by ferulic acid and tannin acid. *Food Hydrocolloids*, 21(4), 575-584.

423 Carvalho, R.A., Sobral, P.J.A., Thomazine, M., Habitante, A.M.Q.B, Giménez, B.,
424 Gómez-Guillén, M.C, & Montero, M.P. (2008). Development of edible films based on
425 differently processed Atlantic halibut (*Hippoglossus hippoglossus*) skin gelatin. *Food*
426 *Hydrocolloids*, 22, 1117–1123.

427 Condés, M. C., Añón, M. C., & Mauri, A. N. (2015). Amaranth protein films prepared
428 with high-pressure treated proteins. *Journal of Food Engineering*, 166, 38–44.

429 Denavi, G. A., Pérez-Mateos, M., Añón, M. C., Montero, P., Mauri, A. N., Gómez-
430 Guillén, M. C. (2009). Structural and functional properties of soy protein isolate and
431 cod gelatin blend films. *Food Hydrocolloids*, 23(8), 2094-2101.

432 Gontard, N., Duchez, C., Cuq, J.L., & Guilbert, S. (1994). Edible composite films of
433 wheat gluten and lipids: water vapour permeability and other physical properties.
434 *International Journal of Food Science and Technology*, 29, 39-50.

435 Govindaraj, P., Kandasubramanian, B., & Kodam, K. M. (2014). Molecular interactions
436 and antimicrobial activity of curcumin (*Curcuma longa*) loaded polyacrylonitrile films.
437 *Materials Chemistry and Physics*, 147, 934-941.

438 Kerry, J. P. (2012). Application of smart packaging systems for conventionally
439 packaged muscle-based food products. In Kerry, J. P. (Ed.). *Advances in Meat, Poultry*
440 *and Seafood Packaging*. Woodhead Publishing Limited, 522-564.

441 Kuswandi, B., Jayus, A. R., Abdullah, A., Heng, L. Y., & Ahmad, M. (2012). A novel
442 colorimetric food package label for fish spoilage based on polyaniline film. *Food*
443 *Control*, 25(1), 18–189.

444 Lawhavinit, O. A., Kongkathip, N., & Kongkathip, B. (2010). Antimicrobial activity of
445 curcuminoids from *Curcuma longa L.* on pathogenic bacteria of shrimp and chicken.
446 *Kasetsart Journal—Natural Science*, 44(3), 364–371.

447 Liu, Y., Cai, Y., Jiang, X., Wu, J., & Le, X. (2016). Molecular interactions,
448 characterization and antimicrobial activity of curcumin-chitosan blend films. *Food*
449 *Hydrocolloids*, 52, 564-572.

450 Maniglia, B. C., Domingos, J. R., de Paula, R. L., & Tapia-Blácido, D. R. (2014).
451 Development of bioactive edible film from turmeric dye solvent extraction residue.
452 *LWT - Food Science and Technology*, 56, 269-277.

453 Maniglia, B. C., de Paula, R. L., Domingos, J. R., & Tapia-Blácido, D. R. (2015).
454 Turmeric dye extraction residue for use in bioactive film production: optimization of
455 turmeric film plasticized with glycerol. *LWT - Food Science and Technology*, 64, 1187-
456 1195.

457 Martins, N., Roriz, C. L., Morales, P., Barros, L., & Ferreira, I. C. F. R. (2016). Food
458 colorants: Challenges, opportunities and current desires of agroindustries to ensure
459 consumer expectations and regulatory practices. *Trends in Food Science & Technology*,
460 52, 1-15.

461 Mauri, A. N., & Añón, M. C. (2006). Effect of solution pH on solubility and some
462 structural properties of soybean protein isolate films. *Journal of the Science of Food*
463 *and Agriculture*, 86(7), 1064–1072.

464 Mayet, N., Kumar, P., Choonara, Y. E., Tomar, L. K., Tyagi, C., du Toit, L.C., & Pillay,
465 V. (2014). Synthesis of a semi-interpenetrating polymer network as a bioactive
466 curcumin film. *AAPS PharmSciTech*, 15(6), 1476-1489.

467 Mehanny, M., Hathout, R. M., Geneidi, A. S., & Mansour, S. (2016). Exploring the use
468 of nanocarrier systems to deliver the magical molecule; curcumin and its derivatives.
469 *Journal of Controlled Release*, 225, 1–30.

470 Moghadamtousi, S. Z., Kadir, H. A., Hassandarvish, P., Tajik, H., Abubakar, S., &
471 Zandi, K. (2014). A review on antibacterial, antiviral, and antifungal activity of
472 curcumin. *BioMed Research International*. http://dx.doi.org/10.1155/2014/186864

473 Musso, Y. S., Salgado, P. R., & Mauri, A. N. (2016). Gelatin based films capable of
474 modifying its color against environmental pH changes. *Food Hydrocolloids*, 61, 523-
475 530.

476 Niamsa N., & C. Sittiwet, C. (2009). Antimicrobial activity of *Curcuma longa* aqueous
477 extract. *Journal of Pharmacology and Toxicology*, 4(4), 173-177.

478 Nur Hanani, Z.A., Roos, Y.H., & Kerry, J.P. (2012). Use of beef, pork and fish gelatin
479 sources in the manufacture of films and assessment of their composition and mechanical
480 properties. *Food Hydrocolloids*, 29, 144-151.

481 Priyadarsini, K. I. (2014). The chemistry of curcumin: from extraction to therapeutic
482 agent. *Molecules*, 19, 20091-20112.

483 Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C., & Ramirez-Tortosa, M.
484 C. (2016). Curcumin and health. *Molecules*, 21, 264-286.

485 Ruiz-Capillas, C. & Jiménez-Colmenero, F. (2005). Biogenic Amines in Meat and Meat
486 Products. *Critical Reviews in Food Science and Nutrition*, 44 (7-8), 489-599.

487 Salgado, P. R., Fernández, G. B., Drago, S. R., & Mauri, A. N. (2011). Addition of
488 bovine plasma hydrolysates improves the antioxidant properties of soybean and
489 sunflower protein-based films. *Food Hydrocolloids*, 25(6), 1433–1440.

490 Salgado, P. R., López-Caballero, M. E., Gómez-Guillén, M. C., Mauri, A. N., &
491 Montero, M. P. (2012). Exploration of the antioxidant and antimicrobial capacity of two
492 sunflower protein concentrate films with naturally present phenolic compounds. *Food*
493 *Hydrocolloids*, 29(2), 374–381.

494 Salgado, P. R., López-Caballero, M. E., Gómez-Guillén, M. C., Mauri, A. N., &
495 Montero, M. P. (2013). Sunflower protein films incorporated with clove essential oil
496 have potential application for the preservation of fish patties. *Food Hydrocolloids*,
497 33(1), 74-84.

498 Salgado, P.R., Molina Ortiz, S.E., Denavi, G.A, Bosch, M.A., Añón, M.C., & Mauri,
499 A.N. (*in press*). Influence of protein conformation on the properties of soybean protein
500 edible films, in Kumar Thakur, V., Kumari Thakur, M., & Kessler, M.R. (Eds.), *Soy-*
501 *Based Bioplastics*, UK Publisher.

502 Wang, Y.J., Pan, M.H., Cheng, A.L., Lin, L.I., Ho, Y.S., Hsieh, C.Y., & Lin, J.K.
503 (1997). Stability of curcumin in buffer solutions and characterization of its degradation
504 products. Journal of Pharmaceutical and Biomedical Analysis, 15(12), 1867-1876.

Table 1. Film nomenclature and final formulation of film-forming dispersions.

Film nomenclature	Final formulation of film-forming dispersions				
	Gelatin (% w/v)	**Glycerol (% w/v)**	**Curcumin (% w/v)**	**pH**	**Solvent**
Gw6	5	1.25	-	6	Water
Gw11	5	1.25	-	11	Water
GCw6	5	1.25	0.02	6	Water
GCw11	5	1.25	0.02	11	Water
Gew6	5	1.25	-	6	Ethanol-water mixture*
Gew11	5	1.25	-	11	Ethanol-water mixture*
GCew6	5	1.25	0.02	6	Ethanol-water mixture*
GCew11	5	1.25	0.02	11	Ethanol-water mixture*

* Ethanol-water mixture (1:1 v/v).

Table 2. CIE-Lab color parameters (L^*, a^* and b^*) and total color difference (ΔE^*) of gelatin-based films (G) and those with curcumin added (GC) obtained with different solvents –water (w) and an ethanol-water mixture (ew)– at different pH (6 and 11).

Film	L^*	a^*	b^*	ΔE^*
Gw6	93.3 ± 0.5^{e}	-0.94 ± 0.07^{a}	2.7 ± 0.6^{a}	1.8 ± 0.5^{a}
Gw11	93.9 ± 0.6^{ef}	-1.07 ± 0.07^{a}	2.0 ± 0.1^{a}	2.6 ± 0.2^{a}
GCw6	89.1 ± 0.4^{d}	-2.40 ± 0.20^{a}	39.3 ± 1.7^{d}	38.3 ± 1.7^{c}
GCw11	83.1 ± 3.3^{c}	7.70 ± 0.20^{b}	25.1 ± 0.9^{c}	27.9 ± 0.8^{b}
Gew6	96.2 ± 0.7^{ef}	-0.70 ± 0.10^{a}	5.4 ± 0.3^{a}	3.5 ± 0.7^{a}
Gew11	97.3 ± 0.3^{f}	-0.40 ± 0.01^{a}	2.4 ± 0.5^{a}	3.4 ± 0.3^{a}
GCew6	72.6 ± 3.4^{b}	21.3 ± 3.00^{c}	86.4 ± 6.0^{e}	90.5 ± 4.3^{e}
GCew11	28.0 ± 2.2^{a}	35.8 ± 3.70^{d}	19.1 ± 1.9^{b}	80.3 ± 6.1^{d}

Reported values for each gelatin film are means ± standard deviation (n=9). Different letters in the same column indicate significant differences between samples ($p<0.05$), according to Tukey´s test.

Table 3. Thickness, moisture content (MC), water solubility (WS) and water vapor permeability (WVP) of gelatin-based films (G) and those with curcumin added (GC) obtained with different solvents –water (w) and an ethanol-water mixture (ew)– at different pH (6 and 11).

Film	Thickness (µm)	MC (%)	WS (%)	WVP*10^{10} (g H_2O/Pa.s.m)
Gw6	51.0 ± 2.3^{abc}	22.1 ± 0.6^{e}	37.6 ± 2.7^{ab}	6.5 ± 0.3^{b}
Gw11	47.8 ± 3.4^{a}	21.4 ± 0.2^{de}	34.6 ± 1.8^{ab}	7.9 ± 0.4^{c}
GCw6	49.6 ± 5.2^{ab}	18.9 ± 0.5^{bc}	30.3 ± 4.7^{a}	1.0 ± 0.01^{a}
GCw11	55.8 ± 3.5^{cde}	20.2 ± 0.3^{cde}	33.8 ± 4.3^{ab}	0.9 ± 0.03^{a}
Gew6	62.4 ± 3.5^{f}	16.9 ± 0.7^{ab}	36.8 ± 0.6^{ab}	1.1 ± 0.3^{a}
Gew11	61.4 ±1.9^{ef}	16.6 ± 0.3^{a}	35.7 ± 3.4^{ab}	1.2 ± 0.3^{a}
GCew6	54.9 ± 5.8^{bcd}	19.4 ± 1.8^{cd}	41.2 ± 3.2^{b}	1.2 ± 0.08^{a}
GCew11	58.2 ± 5.9^{def}	21.3 ± 0.6^{de}	39.3 ± 2.2^{b}	1.2 ± 0.4^{a}

Reported values for each gelatin film are means ± standard deviation (n=9 for thickness, n=3 for MC, WS and WVP). Different letters in the same column indicate significant differences between samples ($p<0.05$), according to Tukey´s test.

Table 4. Tensile strength (TS), elongation at break (EAB), and Young´s modulus (YM), of gelatin-based films (G) and those with curcumin added (GC) obtained with different solvents –water (w) and an ethanol-water mixture (ew)– at different pH (6 and 11).

Film	TS (MPa)	EAB (%)	YM (MPa)
Gw6	3.4 ± 0.3^{c}	159.2 ± 5.5^{ab}	0.15 ± 0.07^{c}
Gw11	4.6 ± 0.1^{d}	206.9 ± 6.0^{d}	0.13 ± 0.09^{bc}
GCw6	3.4 ± 0.6^{c}	157.4 ± 11.4^{ab}	0.06 ± 0.005^{ab}
GCw11	1.9 ± 0.5^{ab}	176.5 ± 20.0^{bc}	0.008 ± 0.001^{a}
Gew6	2.6 ± 0.2^{b}	163.3 ± 13.1^{ab}	0.06 ± 0.01^{ab}
Gew11	1.7 ± 0.3^{a}	175.5 ± 17.9^{bc}	0.01 ± 0.001^{a}
GCew6	1.9 ± 0.1^{ab}	144.3 ± 7.9^{a}	0.02 ± 0.001^{a}
GCew11	3.4 ± 0.6^{c}	198.6 ± 14.9^{cd}	0.007 ± 0.001^{a}

Reported values for each gelatin film are means ± standard deviation (n=12). Different letters in the same column indicate significant differences between samples ($p<0.05$), according to Tukey´s test.

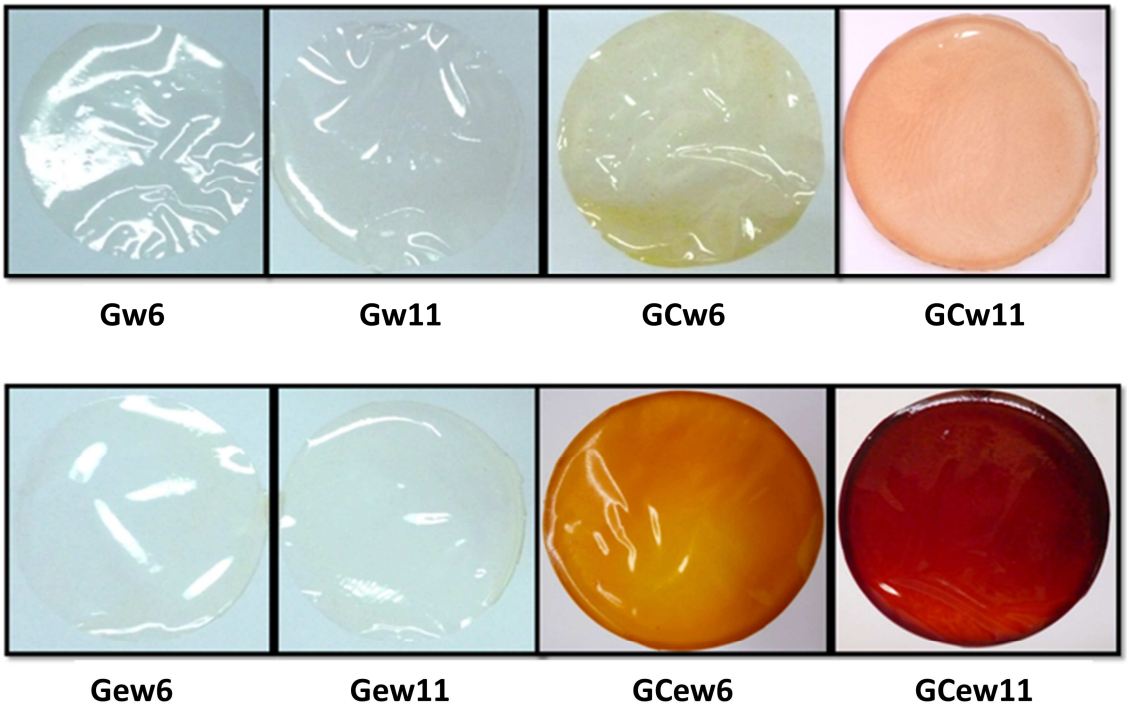


Figure 1. Appearance of gelatin-based films (G) and those with curcumin added (GC) obtained by casting using water (w) or an ethanol-water mixture (ew) as solvent, at pH 6 and 11.

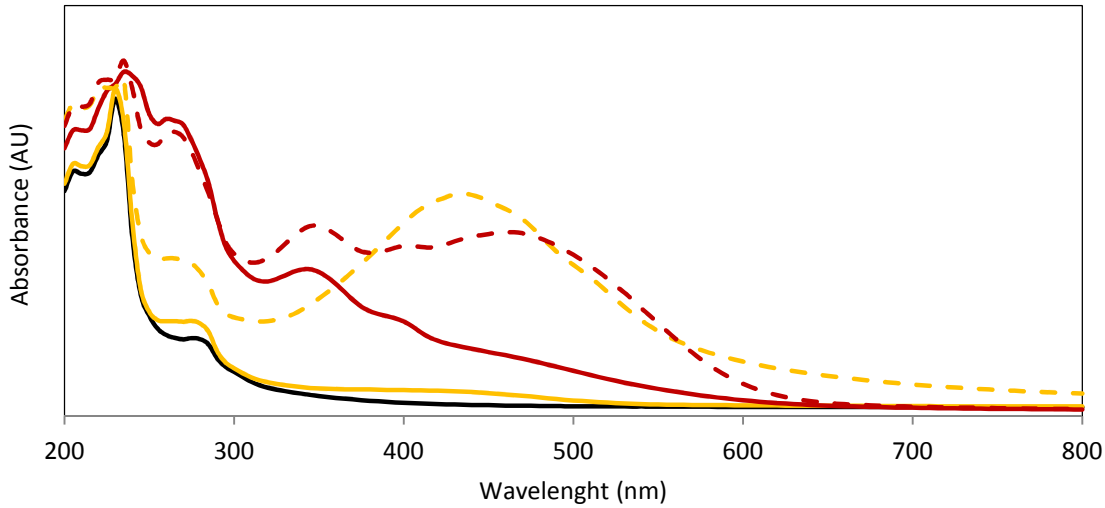


Figure 2. UV-Visible absorption spectra (200-800 nm) of gelatin-based films (G) and those with curcumin added (GC) obtained using water (w) or an ethanol-water mixture (ew) as solvent, at different pH (6 and 11). Spectra of gelatin-based films without curcumin are superimposed: (**——**) **Gw6**, **Gw11**, **Gew6** and **Gew11.** Spectra of gelatin films with curcumin added: (——) **GCw6**; (——) **GCw11**; (**---**) **GCew6** and (**---**) **GCew11**.

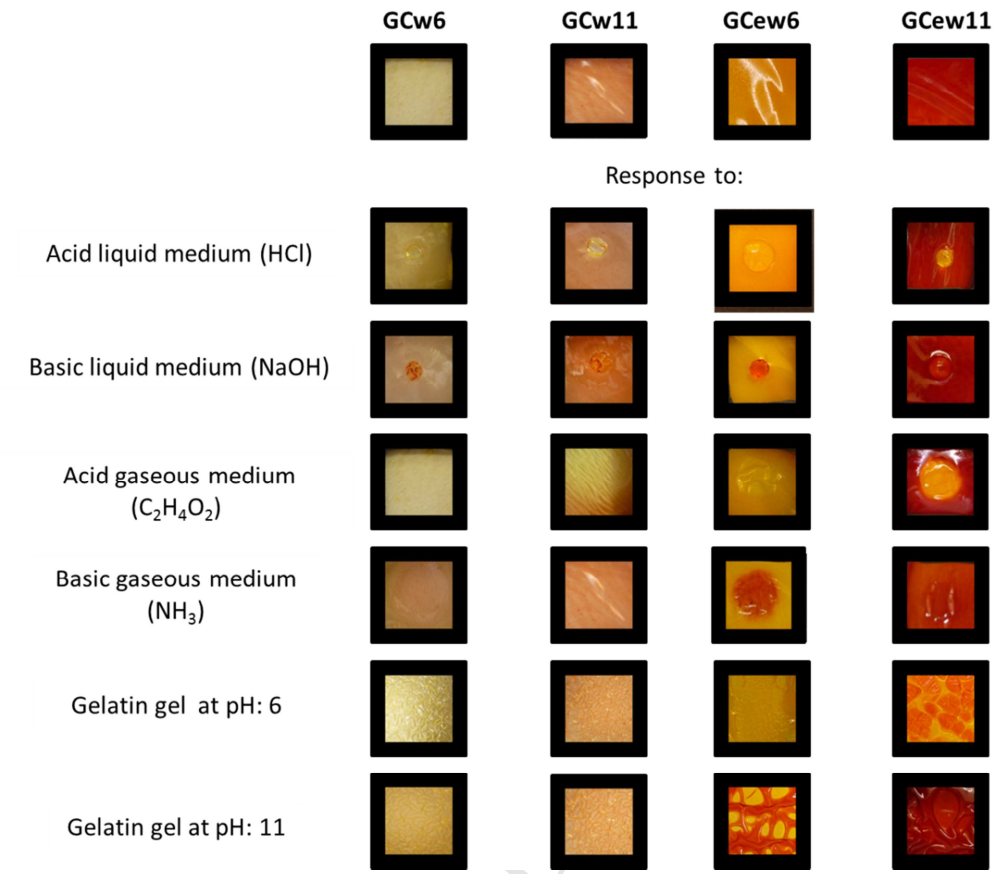


Figure 3. Response of gelatin-based films with curcumin added prepared from aqueous (**GCw6** and **GCw11**) and hydroalcoholic (**GCew6** and **GCew11**) film-forming dispersions at pH 6 and 11 after being in contact with liquid, gaseous and semisolid media of different pH.

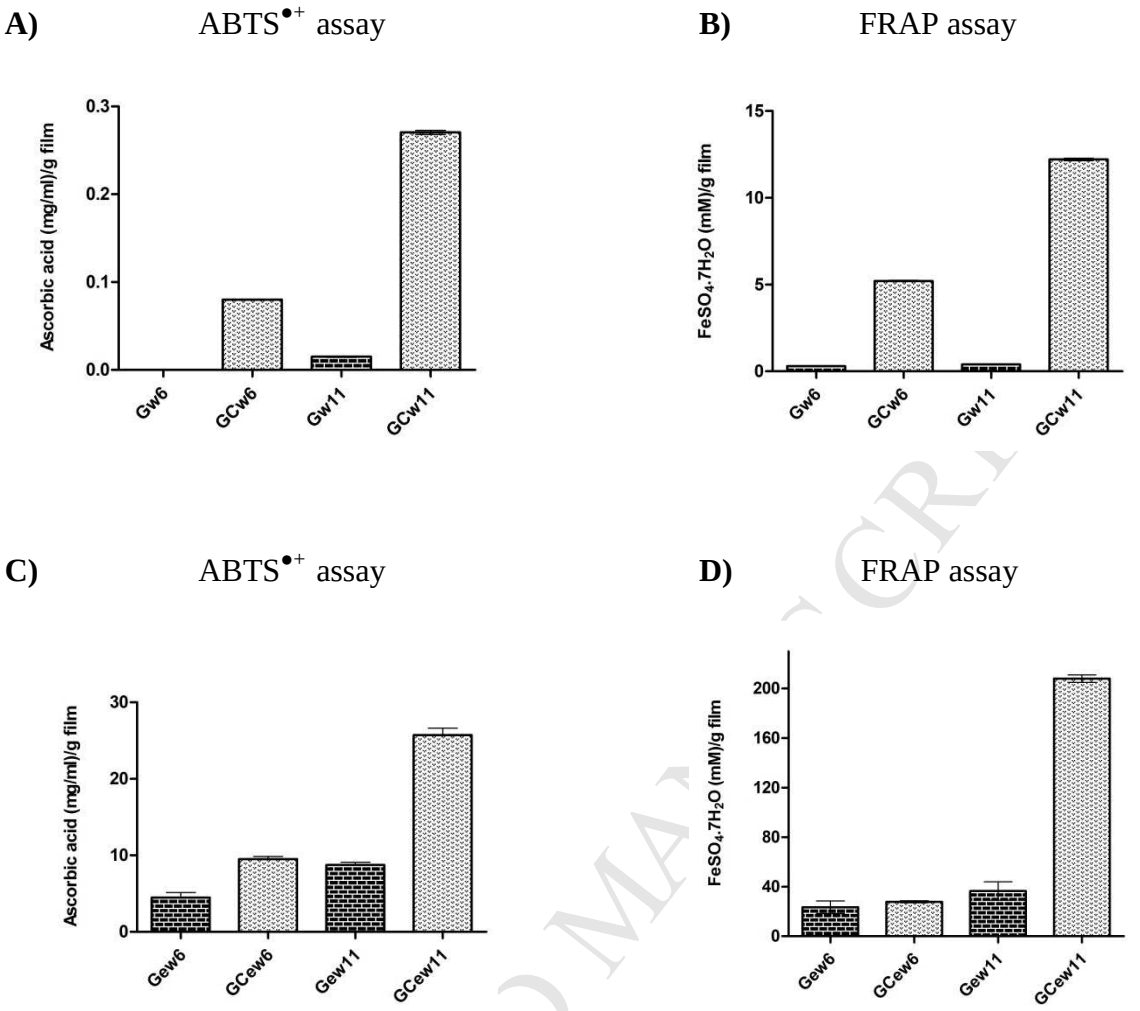


Figure 4. Antioxidant properties (measured by ABTS and FRAP assays) of gelatin-based films (G) and those with curcumin added (GC) obtained with different solvents: water (w) –A and B panels– and an ethanol-water mixture (ew) –B and D panels–, at different pH (6 and 11).

Highlights

- Edible smart gelatin films added with curcumin were developed
- Films modified their color after being in contact with media at different pH
- Curcumin provided films with antioxidant properties but no antimicrobial activity
- Response of films to pH changes were improved using an ethanol-water mixture as solvent
- The use of an ethanol-water mixture as solvent improved films antioxidant properties