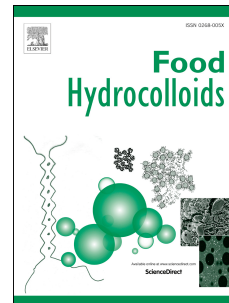


Accepted Manuscript

Gelatin based films capable of modifying its color against environmental pH changes

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



PII: S0268-005X(16)30262-4

DOI: [10.1016/j.foodhyd.2016.06.013](https://doi.org/10.1016/j.foodhyd.2016.06.013)

Reference: FOOHYD 3467

To appear in: *Food Hydrocolloids*

Received Date: 6 March 2016

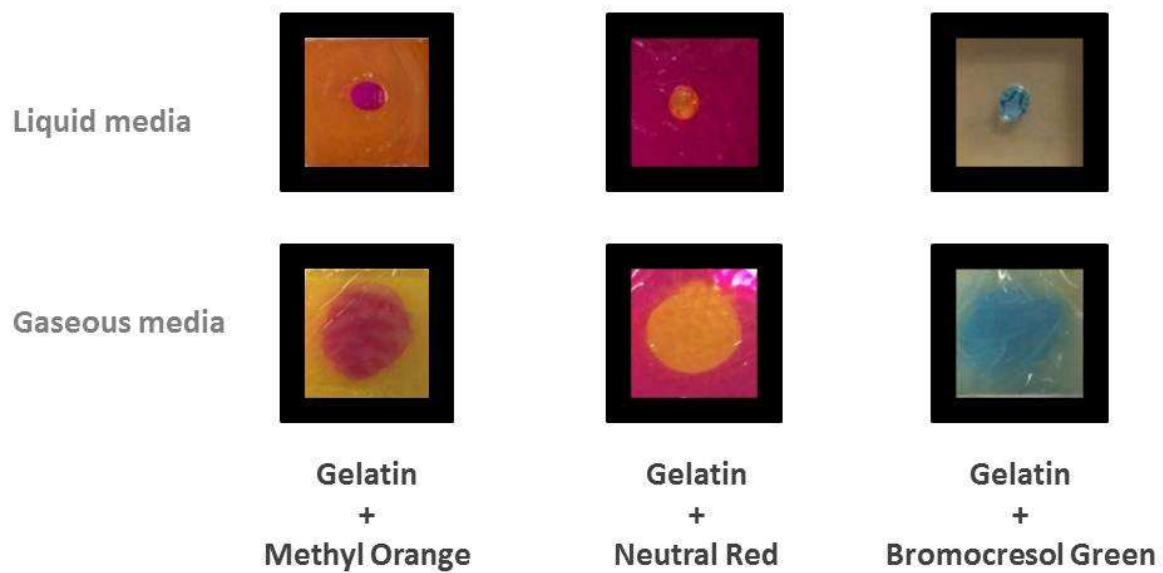
Revised Date: 7 June 2016

Accepted Date: 8 June 2016

Please cite this article as: Musso, Y.S., Salgado, P.R., Mauri, A.N., Gelatin based films capable of modifying its color against environmental pH changes, *Food Hydrocolloids* (2016), doi: 10.1016/j.foodhyd.2016.06.013.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Gelatin films added with acid-base indicators modify their color when being in contact with media of different pH:



ACCEPTED MANUSCRIPT

1 **Gelatin based films capable of modifying its color against environmental pH**
2 **changes**

3
4 Yanina S. Musso, Pablo R. Salgado and Adriana N. Mauri*

5
6 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA),
7 CONICET CCT La Plata y Facultad de Ciencias Exactas, Universidad Nacional de La
8 Plata, 47 y 116 S/N°, (B1900JJ) La Plata, República Argentina.

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

11
12 *Author to whom correspondence should be addressed: Tel.: +54-221-4249287; fax:
13 +54-221-4254853. E-mail: anmauri@quimica.unlp.edu.ar

16 **Abstract**

17 The aim of this work was to develop biodegradable protein-based films capable of sense
18 pH changes. These protein films were prepared by casting from aqueous solutions of
19 bovine gelatin, glycerol and three acid-base indicators: methyl orange (MO), neutral red
20 (NR) and bromocresol green (BCG), at pH 2, 6 and 11. All resulting protein films were
21 homogeneous, thin and had different colors depending on pH and the indicator used.
22 The response of these materials was evaluated simulating their contact with liquid and
23 semisolid media, and with a container headspace at acid and alkaline pH. In all tests,
24 developed protein films could modify their color after being in contact with media of
25 different pH. The physicochemical properties of films were also affected differently by
26 the presence of each acid-base indicator. While the addition of BCG did not
27 significantly modify the properties of control gelatin films, except its color; the
28 incorporation of MO and NR into film-forming solutions significantly improved
29 mechanical properties and decreased the water solubility and moisture content of the
30 resulting protein films without affecting their water vapor permeability.

31

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

33 1. Introduction

34 Innovations in food packaging technologies include the development of new active and
35 smart materials as well as the use of biopolymers as raw materials. These packaging
36 technologies attempts to ensure and extend the safety and quality of products during
37 shelf-life without affecting the environment, in response to new consumers` demands
38 (Brody, Bugusu, Han, Sand, & McHugh, 2008; Dainelli, Gontard, Spyropoulos,
39 Zondervan-van den Beuken, & Tobback, 2008; Restuccia et al., 2010).

40 Biopolymers-based systems can act as carriers of different types of additives. Thus,
41 numerous active packaging systems containing natural or synthetic antioxidant or
42 antimicrobials compounds, ethylene or oxygen captors, probiotics, flavors, etc., has
43 been developed (Campos, Gerschenson, & Flores, 2011; Mellinas et al., 2015; Salgado,
44 Ortiz, Musso, Di Giorgio, & Mauri, 2015; Silva-Weiss, Ihl, Sobral, Gómez-Guillén, &
45 Bifani, 2013). However, there are fewer studies on the development of smart systems
46 capable of monitoring the quality of the packaged food. They often attempt to sense
47 environmental changes or specific compounds generated during food packaging or
48 storage, in order to inform the freshness or microbiological quality of food to
49 manufacturers, retailers or consumers (Biji, Ravishankar, Mohan, & Srinivasa Gopal,
50 2015). Usually these smart devices provide qualitative information through visual
51 colorimetric changes and may be incorporated into the packaging materials or attached
52 to the inside or outside of the package (Ahvenainen, 2003; Biji, Ravishankar, Mohan, &
53 Srinivasa Gopal, 2015; Han & Scanlon, 2005; Kerry, 2008).

54

55 In this regard, the addition of synthetic acid-base indicators (bromocresol green, neutral
56 red, phenol red, bromocresol purple, cresol red, phenolphthalein, bromothymol blue,
57 xylenol blue, p-naphthol-benzene, hexamethoxy red, and their combinations) into
58 polymeric matrices such as polyvinyl alcohol, cellulose acetate, polyethylene and
59 polyethylene terephthalate has been studied by several authors to determine volatile
60 amines, CO₂, SO₂ and other byproducts of bacterial growth (Booher & Gorski, 2011;
61 Eagland, 2004; Gorski & Booher, 2011; Pacquit et al., 2006, 2007). The above-
62 mentioned indicators have been used as model systems since they are not GRAS
63 compounds, but recently some natural compounds, such as grape, flowers and spinach
64 extracts or anthocyanins have been proved to be capable to react to external pH stimuli
65 (Maciel, Yoshida, & Franco, 2015; Veiga-Santos, Ditchfield, & Tadini, 2011; Zhang,
66 Lu, & Chen, 2014).

67 Even though many plant and animal proteins have been used as raw material for
68 producing active packaging (Campos, Gerschenson, & Flores, 2011; Mellinas et al.,
69 2015; Salgado, Ortiz, Musso, Di Giorgio, & Mauri, 2015; Silva-Weiss, Ihl, Sobral,
70 Gómez-Guillén, & Bifani, 2013; Mauri & Añón, 2012; Mauri, Salgado, Condés, &
71 Añón in press), as far as we know, there is no literature related to the formation of pH
72 colorimetric indicator films based on proteins.

73 Proteins are heteropolymers of α -amino acids which differ in their side groups. As they
74 can act as buffer systems due to their ionizable side groups, their film's responsiveness
75 to pH changes is uncertain. Moreover, the aminoacids' side groups could be highly
76 reactive against potential cross-linking or chemical grafting (Guilbert & Gontard, 2005).
77 This potential reactivity could inactivate additives added to the formulation to provide a
78 new functionality, or change protein network cross-linking, thus affecting the
79 physicochemical properties of films.

80 In this context, the aim of the present work was to develop protein films capable of
81 sensing pH changes through the addition of acid-base indicators to film formulations.
82 Gelatin was selected as protein source since their films are colorless (Gómez-Guillén et
83 al., 2009) – unlike plant protein based films which generally present certain color,
84 inherent to non-protein compounds extracted together with proteins (Salgado, Molina
85 Ortiz, Petruccelli, & Mauri, 2010). This colorless would allow films to take the
86 indicator color without interference. Three synthetic acid-base indicators, with different
87 chemical structure and significant color variations in a wide pH range, were selected as
88 system models to activate protein films.

89

90 **2. Material and Methods**

91

92 **2.1 Materials**

93 Bovine gelatin with 240 Bloom (Kraft Foods, Argentina) was used as protein source. Its
94 protein content, as measured by the Kjeldahl method (AOAC, 1995), was 87.8±0.6%
95 (w/w, dry weight; N×5.5). Glycerol (Anedra, Argentina) was used as film plasticizer.
96 Three acid-base indicators were employed: methyl orange (MO, Benzenesulfonic acid,
97 4-[[[(4-dimethylamino)phenyl]azo]-, sodium salt, Mallinckrodt Baker, USA), neutral red
98 (NR, 2,8-Phenazinediamine,N8,N8,3-trimethyl-, monohydrochloride, Pablo Zubizarreta
99 Ward, Argentina) and bromocresol green (BCG, Phenol, 4,4'-(2,2-dioxido-3H-1,2-
100 benzoxathiol-3-ylidene)bis[2,6-dibromo-3-methyl], monosodium salt, Anedra,
101 Argentina). **Table 1** shows their chemical structures, pKa values, pH dependence color,
102 and λ_{max} in the visible region (Sabnis, 2007). All the other reagents used in this study
103 were of analytical grade.

104

105 **2.2 Films preparation**

106 Films were prepared by casting. Initially two aqueous solutions were prepared by
107 magnetic stirring, one containing 10% (w/v) gelatin at 100°C and the other containing
108 2.5 % (w/v) glycerol plus 0.04% (w/v) methyl orange, neutral red or bromocresol green
109 (MO, NR and BCG respectively) at room temperature. Equal volumes of both solutions
110 were then mixed by stirring for additional 30 min at room temperature and the pH was
111 adjusted to 2, 6 and 11, with 2 mol/L HCl or 2 mol/L NaOH. Finally, 10 mL of each
112 film-forming solution were cast onto polystyrene Petri dishes (64 cm²) and dried in an
113 oven with air flow circulation (Yamato, DKN600, USA) at 60°C for 3 h. Resulting films
114 were preconditioned 48 h at 20°C and 58% relative humidity (in desiccators with
115 saturated solutions of NaBr) just before being peeled from the casting surface and
116 characterized.

117 Furthermore, control gelatin films without the incorporation of acid-base indicators into
118 film-forming solutions, at pH= 2, 6 and 11, were obtained as described previously.

119 Three independent batches for each type of protein film (G, G+MO, G+NR, and
120 G+BCG) were performed.

121

122 **2.3 Films characterization**

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

128 *Moisture content (MC)*: Small specimens of films were collected after conditioning, cut
129 and weighed before and after oven drying at 105°C for 24 h, ASTM D644-99, (ASTM
130 2004). MC values were determined in triplicate for each film, and calculated as the
131 percentage of weight loss relative to the original weight.

132 *Color*: Film color was determined with a Konica Minolta Chroma Meter CR-400
133 (Konica Minolta Chroma Co., Osaka, Japan) set to C illuminant/2° observer. A CIE-Lab
134 color scale was used to measure the degree of lightness (L^*), redness ($+a^*$) or greenness
135 ($-a^*$), and yellowness ($+b^*$) or blueness ($-b^*$) of the films. The instrument was
136 calibrated using a white standard plate with color coordinates of $L^*_{standard} = 97.55$,
137 $a^*_{standard} = -0.03$ and $b^*_{standard} = 1.73$ provided by Minolta. Films color was measured on
138 the surface of this standard plate and total color difference (ΔE^*) was calculated as
139 follow:

$$140 \quad \Delta E^* = [(L^*_{film} - L^*_{standard})^2 + (a^*_{film} - a^*_{standard})^2 + (b^*_{film} - b^*_{standard})^2]^{0.5} \quad (1)$$

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

143 *Visible absorption spectra*: Each film specimen was cut into a rectangular piece and
144 placed directly in a spectrophotometer test cell. A spectrum (from 400 to 800 nm) of
145 each film was obtained in an UV-Vis spectrophotometer (Beckman DU650, Germany).
146 Measurements were performed using air as reference. All determinations were
147 performed in triplicate.

148 *Water vapor permeability (WVP)*: Water vapor permeability tests were conducted
149 according to ASTM method E96-00 (ASTM, 2004) with some modifications. Each film
150 sample was sealed over a circular opening of 0.00185 m² in a permeation cell that was
151 stored at 20°C in desiccators. To maintain a 75% relative humidity (RH) gradient across

152 the film, anhydrous silica (0% RH_c) was placed inside the cell and a saturated NaCl
 153 solution (75% RH_d) was used in the desiccators. The RH inside the cell was always
 154 lower than outside, and water vapor transport was determined from the weight gain of
 155 the permeation cell. When steady-state conditions were reached (about 1 h), eight
 156 weight measurements were made over 5 h. Changes in the weight of the cell were
 157 recorded and plotted as a function of time. The slope of each curve ($\Delta m/\Delta t$, g H₂O s⁻¹)
 158 was obtained by linear regression and the water vapor transmission rate (WVTR) was
 159 calculated from the slope divided by the permeation cell area (A, in m²). WVP (g H₂O
 160 Pa⁻¹ s⁻¹ m⁻¹) was calculated as:

$$161 \quad \text{WVP} = [\text{WVTR} / (P_v^{\text{H}_2\text{O}} \cdot (\text{RH}_d - \text{RH}_c))] \cdot d \quad (2)$$

162 Where: WVTR = water vapor transmission rate (g H₂O s⁻¹ m⁻²), P_v^{H₂O} = saturation
 163 water vapor pressure at test temperature (2339.27 Pa at 20 °C), RH_d - RH_c = relative
 164 humidity gradient across the film -expressed as a fraction- (0.75), A = permeation area
 165 (m²), and d = film thickness (m). Each WVP value represents the mean value of three
 166 samples taken from different films.

167 *Water solubility (WS)*: WS was determined as was described by Gontard, Duchez, Cuq,
 168 & Guilbert (1994) with slight modifications. Three pieces of films were weighed
 169 (diameter = 2 cm; ~0.03-0.05 g) and immersed in 50 mL of distilled water. The system
 170 was sealed, shaken at 100 rpm for 24 h at 20°C (Ferca, TT400 model, Argentina), and
 171 then filtered through Whatman n°1 filter paper (previously dried and weighed) to
 172 recover the remaining undissolved film, which was desiccated at 105°C for 24 h. WS
 173 was calculated as follows:

$$174 \quad \text{WS} = [(P_0 \cdot (100 - \text{MC})) - P_f] \cdot 100 / [P_0 \cdot (100 - \text{MC})] \quad (3)$$

175 Where P₀ = initial film weight (g), P_f = final dry film weight (g), MC = moisture content
 176 (%). All tests were carried out in triplicate.

177 *Glass transition temperature (T_g):* T_g was determined by differential scanning
178 calorimetry, using a DSC TA 2010 calorimeter Q100 V9.8 Build 296 (TA Instrument,
179 New Castle, Del., USA) controlled by a TA 5000 module with a quench cooling
180 accessory. Temperature and heat flow calibration of the equipment were carried out
181 according to ASTM methods, using lauric and stearic acids and indium as standards.
182 Hermetically sealed aluminum pans containing 5 mg of films were prepared, and the
183 capsules were scanned at 10°C/min over the range -80 to 150°C. T_g, defined as the
184 inflexion point of the base line, caused by the discontinuity of the specific heat of the
185 sample (ASTM D3418-03 (ASTM, 2004)), was calculated using the Universal Analysis
186 V4.2E software (TA Instruments, New Castle, Del., USA). All the assays were
187 performed at least in duplicate.

188 *Mechanical properties:* Tensile strength (TS), elastic modulus (EM) and elongation at
189 break (EAB) of films were determined following the procedures outlined in the ASTM
190 method D882-02 (ASTM, 2004), using a texture analyzer TA.XT2i (Stable Micro
191 Systems, Surrey, England) equipped with a tension grip system A/TG. Films probes of
192 90 mm length and 6 mm width were used. The initial grip separation was set at 50 mm
193 and the crosshead speed at 0.4 mm s⁻¹. Measurements were made at 20°C in a
194 temperature-controlled room.

195 The curves of force (N) as a function of distance (mm) were recorded by the Texture
196 Expert V.1.15 software (Stable Micro Systems, Surrey, England). Tensile properties
197 were calculated from the plot of stress (tensile force/initial cross-sectional area) versus
198 strain (extension as a percentile of the original length). TS and EAB were determined
199 directly from the stresses-strain curves, and EM was determined as the slope of the initial
200 linear portion of this curve. Reported values are the average of at least twelve
201 replications taken from different films for each formulation.

202 **2.4 Films' response to pH changes**

203 Each film was faced with liquid, semisolid and gaseous media of different pH: i) adding
204 a drop of 2 mol/L HCl or 2 mol/L NaOH directly on films; ii) placing the films in
205 contact with gels prepared from gelatin solutions at 7.5% w/v at pH= 2.5, and 11; and
206 iii) exposing the films to gaseous atmospheres generated by acetic acid glacial ($C_2H_4O_2$,
207 $pK_a \sim 4.8$, Anedra, Argentina) and ammonia (NH_3 , $pK_a \sim 9.3$, Anedra, Argentina).
208 Photographs of films before and after (30 minutes) contacting it with those media of
209 different pH were taken with a digital camera (Kodak M853, USA) and color variations
210 were measured using a colorimeter (Konica Minolta Chroma Meter CR-400), as
211 described above, ~~at the same time films were photographed.~~

212

213 **2.5 Statistical analysis**

214 Results were analyzed by two-way ANOVA (two factors: pH and presence of acid-base
215 indicator, in three and four levels, respectively: pH=2, 6 and 11; control films (G) and
216 those added with MO, NR and BCG (G+MO, G+NR and G+BCG, respectively). Means
217 were tested with the Tukey's HSD (honestly significant difference) test for paired
218 comparison, with a significance level $\alpha=0.05$, using the Statgraphics Plus version 5.1
219 software (Statgraphics, USA).

220

221 **3. Results and Discussion**

222 *3.1 Appearance and optical properties of films*

223 All gelatin films prepared with or without methyl orange, neutral red and bromocresol
224 green acid-base indicators at pH 2, 6 and 11 were homogeneous, thin, flexible, and
225 transparent. **Figure 1** shows their visual appearance. Control gelatin films (G) were
226 clear and colorless for all pHs tested. The addition of methyl orange (MO), neutral red

227 (NR) and bromocresol green (BCG) to film-forming solutions allowed to obtain
228 transparent films with different and well defined colors, dependent on the nature of each
229 acid-base indicator and the solutions pH (2, 6, and 11). Even the color of films matches
230 to the inherent color of the indicators at each pH, reported in **Table 1**. Color parameters
231 (L^* , a^* , b^* and ΔE^*) and the absorption spectra in the visible range of protein films are
232 shown in **Table 2** and **Figure 2** respectively. Regardless of the pH of the film-forming
233 solutions, control gelatin films (**G**) showed a high brightness (high L^*), absence of color
234 (low values of a^* , b^* , and ΔE^*) ($p > 0.05$), and no signal in their absorption spectra in
235 the visible range (data not shown). But these protein films acquired a specific coloration
236 with the addition of the acid-base indicators to the formulations, characterized by
237 different values of a^* and b^* , and a significant lower brightness than **G** films ($p < 0.05$).
238 The absorption spectra of these colored films showed peaks at different wavelengths in
239 the visible range, which were related to their colorations. Gelatin films incorporated
240 with MO (**G+MO**) were orange at pH 2, yellow at pH 6, and purple at pH 11, with
241 maximum absorptions (λ_{\max}) at 510 nm, 430 nm, and 570 nm in their respective spectra
242 (**Figure 2.A**). It is worth noting that films with MO at alkaline pH showed a purple
243 color not reported for this indicator in the cited literature (Sabnis, 2007). On the other
244 hand, gelatin films incorporated with NR (**G+NR**) were yellow at pH 11, and purple at
245 $\text{pH} \leq 6$, with λ_{\max} at 460 nm and 520 nm in their respective visible spectra (**Figure 2B**).
246 But it is possible to note, that those films prepared at pH 2 showed a higher absorption
247 peak and a higher intensity of the hue (with higher values of a^* and lower values of b^*)
248 than those prepared at pH 6. Finally, gelatin films incorporated with BCG (**G+BCG**)
249 were barely yellow at pH 2 and blue at pH 6 and 11, with maximum absorptions at 440
250 nm and 620 nm respectively (**Figure 2C**). For this indicator, films at pH 11 showed a

251 more intense coloration than those at pH 6, evidenced by an increase in its absorption
252 peak, a more negative b^* value and a higher a^* value.

253 Coloration of films could be considered as an additional attribute for some commercial
254 applications. These materials can act as barriers to visible light, protecting food
255 products from oxidation (Cian, Salgado, Drago, González, & Mauri, 2014).

256

257 *3.2 Films' response to pH changes*

258 **Figure 3** shows the response of all developed films when placed in contact with acid
259 and alkali liquids, semisolids and gases. This assay allows verifying the ability of these
260 films to sense pH changes, simulating that these changes could occur in a liquid or
261 semisolid food, or in the headspace of a food container as the result of the reaction
262 products of food spoilage. Thus, the material could inform indirectly about the quality
263 and safety of the product during its storage and distribution chain until be consumed.

264 All color changes seen in **Figure 3**, which were reversible, were confirmed by
265 colorimetric measurements. Hunter color parameters L^* , a^* and b^* are shown as
266 supplementary material.

267 Gelatin films incorporated with MO, NR, and BCG could change their color after being
268 in contact with alkali or acid solutions of NaOH or HCl respectively, gaseous
269 atmospheres of acetic acid or ammonia, and gelatin gels at pH 2.5 and 11, except for
270 those in which the pH of the medium and film were similar. These film responses were
271 immediately and markedly with liquid and gases of different pH, but less evident and
272 slower with semisolid media. Slower turning kinetics of acid-base indicators against
273 semisolid media could probably be attributed to limited diffusive processes.

274 **Figure 3.A** shows changes in color of gelatin films incorporated with MO (**G+MO**)
275 after being in contact with different pH media. For example, films obtained at pH 6

276 resulted initially yellow, but became orange or purple by placing a drop of HCl or
277 NaOH solutions on them respectively. The same behavior was observed when the films
278 were exposed to acidic or alkaline gaseous atmospheres. It is noteworthy that acidic
279 gaseous atmosphere produced by acetic acid did not alter the color of the yellow film at
280 pH 6 and turned purple to yellow film at pH 11, not reaching the characteristic orange
281 color of MO in acidic medium. This could be attributed to the pKa of acetic acid (pKa ~
282 4.8) that is higher than the pH at which MO turns to its acid form (pKa=3.7).
283 Films at pH 6 and 11 in contact with semisolid medium at pH 2.5 veered to the same
284 yellow acquired by acidic films, instead of the expected orange coloration. This could
285 be attributed to the diffusion of the indicator to the gel during the assay, which also
286 provided color to the media. Meanwhile against semisolid media at pH 11, films at pH 6
287 reached the alkaline purple coloration, but those of pH 2 turned yellow. It seemed that
288 these acid films failed to achieve the pH of the gel or that their structural characteristics
289 favored the diffusion of the indicator, according to the observations previously
290 mentioned.

291 **Figure 3.B** shows how gelatin films with NR in their formulation (**G+NR**) could sense
292 the pH of the surrounding medium. They modified their color by placing a drop of acid
293 or alkali on them or when subjected to acidic or alkaline gaseous atmospheres. As noted
294 above, the changes in films color were less evident when they were contacted with
295 semisolids, at different pHs.

296 Gelatin films incorporated with BCG (**G+BCG**) showed similar behavior than **G+NR**
297 films (**Figure 3.C**). They changed their color clearly and immediately after being in
298 contact with acid and alkaline liquids and gaseous media. These color changes were
299 very noticeable since the films turned from barely yellow (at acidic pH) to blue (at

300 neutral or alkaline pH) or vice versa, being these changes less evident when films were
301 contacted with semisolid media.

302 Microbial growth often influence the pH of the medium due to metabolites produced by
303 microorganisms, for example lactic acid, hydrogen sulfide, volatile amines, etc. (Biji,
304 Ravishankar, Mohan, & Srinivasa Gopal, 2015; Han & Scanlon, 2005; Kerry & Butler,
305 2008). If packaging material could sense this change through a change in its color, it
306 would inform producers, sellers and consumers about the quality and safety of the
307 packaged food (Biji, Ravishankar, Mohan, & Srinivasa Gopal, 2015; Kerry & Butler,
308 2008)

309

310 *3.3 Effect of pH and acid-base indicators addition on the physicochemical properties of* 311 *films*

312 Regardless of the presence of acid-base indicators in formulations, pH of film-forming
313 solutions affects the ionization state and the conformation of proteins, thus conditioning
314 the interactions that can occur between polypeptide chains and among proteins and
315 other components during film formation. Protein–protein interactions involved in film
316 matrix stabilization determine the cross-linking degree and the hydrophilic-
317 hydrophobic character of the films, which correlate with their physicochemical,
318 mechanical, and barrier properties (Mauri & Añón, 2006, 2008). Furthermore, the
319 incorporation of additives into materials formulation attempting to confer specific
320 functionalities on films –such as antioxidants, antimicrobials, vitamins,
321 microorganisms, probiotics, flavors, and pigments– could also affect protein cross-
322 linking and therefore modify the physicochemical properties of the resulting materials
323 (Salgado, Ortiz, Musso, Di Giorgio, & Mauri, 2015; Mauri, Salgado, Condés & Añón
324 in press).

325 Thickness, moisture content (MC), water solubility (WS), water vapor permeability
326 (WVP) and glass transition temperature (T_g) of developed films are showed in **Table 3**.
327 No modification in films thickness (~ 50 μm) was observed with the addition of acid-
328 base indicators used (p>0.05) neither with the pH of the film-forming dispersion
329 (p>0.05). Moisture content of control gelatin films (**G**) –without acid-base indicator
330 addition– were ~20%. The addition of **MO** and **NR** into formulations significantly
331 decreased the moisture content of the resulting films (**G+MO** and **G+NR**) (p<0.05) at
332 all studied pH, while the incorporation of **BCG** did not modify their moisture content
333 respect to **G** films (p>0.05). Variation on pH only modified the moisture content of **G**
334 and **G+BCG** films (p<0.05) slightly. In both cases, films obtained at pH 6 shows the
335 highest MC values (p<0.05).

336 Control gelatin films (**G**) showed interesting water solubilities –between 37 and 49 %
337 depending on the pH of film-forming solutions – which resulted lower than others
338 values reported in the literature for this protein films (Nur Hanani, Roos, & Kerry,
339 2012). The addition of the acid-base indicators into the formulations caused different
340 effects on the water solubility of the resulting films. **MO** provoked a significantly
341 decrease in water solubility of the resulting films (p<0.05), being this effect higher at
342 pH 11 (*ca.* 60%) than at pH 2 and 6 (*ca.* 40%). **NR** did not affect the water solubility of
343 gelatin films (p>0.05) and **BCG** caused differential behaviors on water solubility
344 depending on the pH of the film-forming solutions: increased it ~25% at pH=11
345 (p>0.05), decreased it ~40% at pH=6 (p>0.05), and did not modify it at pH=2 (p<0.05).

346 Control gelatin films and those colored by **MO** and **NR** prepared at acidic pH were
347 more soluble than those obtained at neutral or alkaline pH (p<0.05). But those colored
348 by **BCG** showed similar water solubilities at pH 2 and 11, higher than at pH 6 (p<0.05).

349 These results suggest a different protein cross-linking degree dependent on the presence
350 of the acid-base indicators and pH of film-forming solutions.

351 Unlike water solubility and moisture content results, no significant differences in water
352 vapor permeability (WVP) of films were observed ($\sim 8.2 \cdot 10^{-11} \text{ g H}_2\text{O s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$) with
353 the addition of acid-base indicators ($p > 0.05$) or changing the pH of film-forming
354 solutions ($p > 0.05$).

355 Mechanical properties of developed gelatin films are presented in **Figure 4**. Control
356 gelatin films (**G**) showed moderate tensile strength (TS), Young's modulus (EM), and
357 elongation at break (EAB). These properties were affected by both the presence and
358 type of acid-base indicator ($p < 0.05$) as by the initial pH of protein dispersion ($p < 0.05$).

359 Incorporation of **MO** or **NR** into formulations improved the mechanical properties of
360 these materials. This colored films showed higher tensile strength and Young's modulus
361 but lower elongation at break than control films (**G**) ($p < 0.05$). These improvements
362 were most notable at neutral and alkaline pH than at acidic pH. **G+NR** films showed the
363 best mechanical properties of films developed. In particular, addition of **NR** to gelatin
364 film-forming solutions at pH=11 markedly increased tensile strength (*ca.* 400%) and
365 Young's modulus (*ca.* 2000%) of resulting films ($p < 0.05$), in detriment of its elongation
366 at break (*ca.* 40% decrease) ($p < 0.05$). Moreover, **G+BCG** films had similar mechanical
367 properties than respective control films ($p > 0.05$). And it is worth noting that gelatin-
368 based films added or not with different acid-base indicators obtained at pH 6 and 11
369 showed higher tensile strength than those prepared from acidic film-forming solutions
370 ($p < 0.05$).

371 These results suggest that studied acid-base indicators, with different chemical
372 structures (shown in **Table 1**), could interact differently with gelatin in the protein
373 network. Addition of **MO** and **NR** to formulations seems to favor protein cross-linking,

374 leading to more resistant and less water soluble films, with lower moisture content and
375 without affecting their water vapor permeability. Whereas **BCG** addition seems not
376 interfere in protein matrixes obtained at pH 6 and 11, but favor certain plasticizing
377 effect in acidic films.

378 Glass transition temperatures (T_g) of studied films are presented in **Table 3**. All films
379 showed just one T_g , suggesting that no phase separation was
380 observed (Tapia-Blácido, Mauri, Menegalli, Sobral, and Añón, 2007). Neither the
381 presence and type of acid-base indicators nor pH of the film-forming solutions modified
382 T_g of the materials ($p > 0.05$), except for **G+MO** and **G+BCG** films at pH 6 that showed
383 slightly higher T_g than control films ($p < 0.05$). These results did not represent the
384 greater cross-linking or the possible plasticizing effect on protein matrix suggested
385 above when analyzing moisture content, water solubility and mechanical properties of
386 films. The different moisture content of films also is affecting the T_g value. These
387 results suggest that **MO** and **NR** molecules could be acting as physical and/or chemical
388 entanglements not modifying the mobility of polypeptide chains.

389

390 **4. Conclusions**

391 Gelatin-based films capable of sensing changes in the surrounding pH medium were
392 developed by addition of methyl orange, neutral red and bromocresol green –known
393 acid-base indicators– in their formulation. All films modified its color reversibly when
394 they were in contact with liquid, gaseous and semisolid media of different pHs. The
395 addition of these compounds also modifies the physicochemical properties of the
396 resulting materials. In particular, methyl orange and neutral red could be acting as
397 physical and/or chemical entanglements, increasing the tensile strength and reducing the
398 water solubility of the resulting films, without affecting their water vapor permeability

399 and their capacity to change their color against the pH of the surrounding medium.
400 These smart materials, used as food packaging or coatings, could inform about the
401 safety and quality of any product whose deterioration mode caused a change in the pH
402 of the media, such as microbial growth.

403 Evidence that the protein matrix did not interfere with the discoloration of the acid-base
404 indicators when being in contact with a medium of different pH, pushed to find food
405 grade dyes that could replace the synthetic ones analyzed in this work and to probe this
406 materials as packaging of real systems.

407

408 **Acknowledgements**

409 The authors are thankful to the National Agency of Scientific and Technological
410 Support (PICT-2010-1837, PICT-2013-2124) and the Universidad Nacional de La Plata
411 (11/X618) for their financial support.

412

413 **References**

414 Ahvenainen, R. (2003). *Novel food packaging techniques*. Elsevier Ltd.

415 AOAC.(1995). *Official methods of analysis of AOAC international*. (16th ed.),

416 Horowitz, Washigton DC, USA.

417 ASTM. (2004). *Annual book of ASTM standards*. ASTM International: Philadelphia,

418 PA, USA.

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

- 422 Booher J, Gorski J. R. (2011). Polymeric indicators for detecting the presence of
423 metabolic byproducts from microorganisms. Patent US20110042344.
- 424 Brody, A. L., Bugusu, B., Han, J. H., Sand, C. K., & McHugh, T. H. (2008). Innovative
425 food packaging solutions. *Journal of Food Science*, 73, R107-R116.
- 426 Campos, C., Gerschenson, L., & Flores, S. (2011). Development of edible films and
427 coatings with antimicrobial activity. *Food and Bioprocess Technology*, 4, 849–875.
- 428 Cian, R. E., Salgado, P. R., Drago, S. R., González, R. J., & Mauri, A. N. (2014).
429 Development of naturally activated edible films with antioxidant properties prepared
430 from red seaweed *Porphyra columbina* biopolymers. *Food Chemistry*, 146, 6–14.
- 431 Dainelli, D., Gontard, N., Spyropoulos, D., Zondervan-van den Beuken, E., & Tobback,
432 P. (2008). Active and intelligent food packaging: legal aspects and safety concerns.
433 *Trends in Food Science and Technology*, 19, S103–S112.
- 434 Eagland D. (2004). Polymeric materials incorporating a pH indicator dye. Patent
435 US20070276207.
- 436 Gómez-Guillén, M. C., Pérez-Mateos, M., Gómez-Estaca, J., López-Caballero, E.,
437 Giménez, B., & Montero, P. (2009). Fish gelatin: a renewable material for developing
438 active biodegradable films. *Trends in Food Science & Technology*, 20, 3–16.
- 439 Gontard, N., Duchez, C., Cuq, J., & Guilbert, S. (1994). Edible composite films of
440 wheat gluten and lipids - water-vapor permeability and other physical-properties.
441 *International Journal of Food Science and Technology*, 29, 39–50.

- 442 Gorski J. R., Booher J. (2011). Processes for preparing a polymeric indicator film. Patent
443 US20110274593.
- 444 Guilbert, S., & Gontard, N. (2005). Agro-polymers for edible and biodegradable films.
445 Review of agricultural polymeric materials, physical and mechanical characteristics.
446 *Innovations in Food Packaging*, 263–276.
- 447 Han, J., & Scanlon, M. (2005). Mass transfer of gas and solute through packaging
448 materials. In Han, J. (Ed.). *Innovations in food packaging* (pp. 12–23). Elsevier
449 Academic Press.
- 450 Kato, E. T., Yoshida, C. M., Reis, A. B., Melo, I. S., & Franco, T. T. (2011). Fast
451 detection of hydrogen sulfide using a biodegradable colorimetric indicator system.
452 *Polymer International*, 60, 951–956.
- 453 Kerry J. & Butler, P. (2008). *Smart packaging technologies for fast moving consumer*
454 *goods*. J. Wiley.
- 455 Maciel, V. B. V., Yoshida, C. M. P., & Franco, T. T. (2015). Chitosan/pectin
456 polyelectrolyte complex as a pH indicator. *Carbohydrate Polymers*, 132, 537-545.
- 457 Mauri A. N. & Añón M. C. (2012). Proteínas como envases alimentarios. In Olivas
458 Orozco G.I., González-Aguilar G.A., Martín-Belloso O. and Soliva-Fortuny R.
459 Películas y recubrimientos comestibles: propiedades y aplicaciones en alimentos. (pp.
460 95-124). Editorial Clave.
- 461 Mauri A. N., Salgado P.R., Condés M.C & Añón M.C. (in press). Films and coatings
462 from vegetable proteins. In Montero M.P, Gómez-Guillén M.C., López-Caballero M.E.

- 463 &. Barbosa-Cánovas G.V. (Eds.). Edible films and coatings: Fundamentals and
464 applications. CRC Press, Taylor & Francis Group.
- 465 Mauri, A. N., & Añón, M. C. (2006). Effect of solution pH on solubility and some
466 structural properties of soybean protein isolate films. *Journal of Science of Food and*
467 *Agriculture*, 86, 1064–1072.
- 468 Mauri, A. N., & Añón, M. C. (2008). Mechanical and physical properties of soy protein
469 films with pH-modified microstructures. *Food Science and Technology International*,
470 14, 119–125.
- 471 Mellinas, C., Valdés, A., Ramos, M., Burgos, N., Garrigós, M. del C., & Jiménez, A.
472 (2015). Active edible films: Current state and future trends. *Journal of Applied Polymer*
473 *Science*, 132, 42631.
- 474 Nur Hanani, Z. A., Roos, Y. H., & Kerry, J. P. (2012). Use of beef, pork and fish gelatin
475 sources in the manufacture of films and assessment of their composition and mechanical
476 properties. *Food Hydrocolloids*, 29, 144–151.
- 477 Pacquit, A., Lau, K. T., McLaughlin, H., Frisby, J., Quilty, B., & Diamond, D. (2006).
478 Development of a volatile amine sensor for the monitoring of fish spoilage. *Talanta*, 69,
479 515–20.
- 480 Pacquit, A., Frisby, J., Diamond, D., Lau, K., Farrell, A., Quilty, B., & Diamond, D.
481 (2007). Development of a smart packaging for the monitoring of fish spoilage. *Food*
482 *Chemistry*, 102, 466–470.

- 483 Restuccia, D., Spizzirri, U. G., Parisi, O. I., Cirillo, G., Curcio, M., Iemma, F., & Picci,
484 N. (2010). New EU regulation aspects and global market of active and intelligent
485 packaging for food industry applications. *Food Control*, 21, 1425–1435.
- 486 Sabnis R.W. (2007). *Handbook of acid-base indicators*. CRC Press.
- 487 Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S., & Mauri, A. N. (2010).
488 Biodegradable sunflower protein films naturally activated with antioxidant compounds.
489 *Food Hydrocolloids*, 24, 525–533.
- 490 Salgado, P. R., Ortiz, C. M., Musso, Y. S., Di Giorgio, L., & Mauri, A. N. (2015).
491 Edible films and coatings containing bioactives. *Current Opinion in Food Science*, 5,
492 86–92.
- 493 Silva-Weiss, A., Ihl, M., Sobral, P. J. A., Gómez-Guillén, M. C., & Bifani, V. (2013).
494 Natural additives in bioactive edible films and coatings: functionality and applications
495 in foods. *Food Engineering Reviews*, 5, 200–216.
- 496 Tapia-Blácido D., Mauri A.N., Menegalli F.C., Sobral P.J.A., & Añón M.C. (2007).
497 Contribution of the starch, protein, and lipid fractions to the physical, thermal, and
498 structural properties of amaranth (*Amaranthus caudatus*) flour films. *Journal of Food*
499 *Science*, 72, 293-300.
- 500 Veiga-Santos P., Ditchfield C., Tadini C.C. (2011). Development and evaluation of a
501 novel pH indicator biodegradable film based on cassava starch. *Journal of Applied*
502 *Polymer Science*, 120, 1069-1079.
- 503 Zhang, X., Lu, S., & Chen, X. (2014). A visual pH sensing film using natural dyes from
504 *Bauhinia Blakeana* Dunn. *Sensors and Actuators B: Chemical*, 198, 268–273.

505

506 **Figure legends**

507

508 **Figure 1.** Appearance of control gelatin-based films (G) and those added with methyl
509 orange (G+MO), neutral red (G+NR), and bromocresol green (G+BCG) at pH 2, 6 and
510 11.

511

512 **Figure 2.** Visible absorption spectra (400-800 nm) of gelatin-based films added with
513 methyl orange (**A**), neutral red (**B**) and bromocresol green (**C**) at pH 2, 6, and 11
514 respectively.

515

516 **Figure 3.** Response of gelatin-based films added with methyl orange (G+MO, **A**),
517 neutral red (G+NR, **B**), and bromocresol green (G+BCG, **C**) at pH 2, 6 and 11 after
518 being in contact with liquid, gaseous and semisolid media of different pHs.

519

520 **Figure 4.** Mechanical properties of gelatin-based films obtained at different pH (2, 6
521 and 11) added or not with different acid-base indicators (MO, NR, and BCG). **A**)
522 Tensile strength (TS). **B**) Young's modulus (EM). **C**) Elongation at break (EAB).

523

524 **Table captions**

525

526 **Table 1.** Chemical structure, pKa, pH dependence color, and λ_{max} in the visible region
527 of methyl orange (MO), neutral red (NR) and bromocresol green (BCG), used in this
528 manuscript as pH indicators.

529

530 **Table 2.** CIE-Lab color parameters (L^* , a^* and b^*) and total color difference (ΔE^*) of
531 gelatin-based films added or not with different acid-base indicators (MO, NR, BCG)
532 obtained at different pH (2, 6 and 11).

533

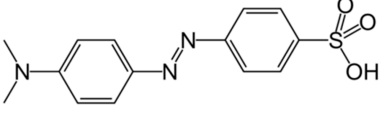
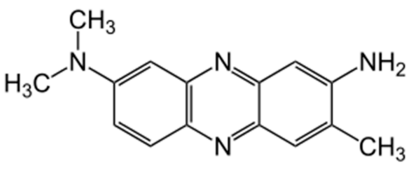
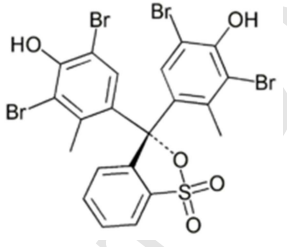
534 **Table 3.** Thickness, moisture content (MC), water solubility (WS), water vapor
535 permeability (WVP) and glass transition temperature (T_g) of gelatin-based films (G)
536 added or not with methyl orange (MO), neutral red (NR), and bromocresol green (BCG)
537 at pH 2, 6 and 11.

538

539 **Supplementary Table.** CIE-Lab color parameters (L^* , a^* and b^*) of gelatin (G) films
540 added with methyl orange (MO), neutral red (NR) and bromocresol green (BCG) at pH
541 2, 6, and 11 and their corresponding responses against acid or alkali liquid, gaseous and
542 semisolid media.

543

Table 1. Chemical structure, pKa, pH dependence color, and λ_{max} in the visible region of methyl orange (MO), neutral red (NR) and bromocresol green (BCG), used in this manuscript as pH indicators (*).

| Acid-base indicator | Chemical structure | λ_{max} | pKa | Color change |
|-------------------------|---|----------------------|-----|------------------------------------|
| Methyl Orange (MO) |  | 507-522 nm 464 nm | 3.7 | Red at pH<3.0 Yellow at pH>4.4 |
| Neutral Red (NR) |  | 529-544 nm 454 nm | 7.4 | Red at pH<6.8 Yellow at pH>8.0 |
| Bromocresol Green (BCG) |  | 423-444 nm 617 nm | 4.6 | Yellow at pH<3.8 Blue at pH>5.4 |

(*). Data from Sabnis [29].

Table 2. CIE-Lab color parameters (L^* , a^* and b^*) and total color difference (ΔE^*) of gelatin-based films added or not with different acid-base indicators (MO, NR, BCG) obtained at different pH (2, 6 and 11).

| Film | pH | L^* | a^* | b^* | ΔE^* |
|-------|----|------------------------|-------------------------|-------------------------|------------------------|
| G | 2 | $94.41 \pm 0.21^{a/x}$ | $-0.79 \pm 0.06^{a/x}$ | $2.10 \pm 0.17^{a/x}$ | $2.06 \pm 0.12^{a/x}$ |
| | 6 | $93.35 \pm 0.55^{a/x}$ | $-0.94 \pm 0.07^{a/x}$ | $2.70 \pm 0.61^{a/x}$ | $1.85 \pm 0.52^{a/x}$ |
| | 11 | $93.87 \pm 0.58^{a/x}$ | $-1.07 \pm 0.07^{a/x}$ | $2.05 \pm 0.11^{a/x}$ | $2.64 \pm 0.22^{b/x}$ |
| G+MO | 2 | $80.30 \pm 0.50^{a/y}$ | $26.50 \pm 0.04^{a/y}$ | $61.70 \pm 1.62^{a/y}$ | $11.8 \pm 0.09^{a/y}$ |
| | 6 | $79.67 \pm 0.28^{a/y}$ | $17.11 \pm 0.26^{b/y}$ | $65.27 \pm 0.08^{b/y}$ | $11.30 \pm 0.05^{b/y}$ |
| | 11 | $47.02 \pm 0.29^{b/y}$ | $58.34 \pm 0.37^{c/y}$ | $-4.17 \pm 0.18^{c/y}$ | $2.41 \pm 0.29^{c/x}$ |
| G+NR | 2 | $50.63 \pm 0.72^{a/z}$ | $58.01 \pm 0.52^{a/z}$ | $4.64 \pm 0.37^{a/x}$ | $5.48 \pm 0.17^{a/z}$ |
| | 6 | $62.69 \pm 0.64^{b/z}$ | $23.12 \pm 0.92^{b/z}$ | $25.85 \pm 0.49^{b/z}$ | $5.18 \pm 0.17^{b/z}$ |
| | 11 | $64.93 \pm 0.98^{c/z}$ | $16.81 \pm 0.85^{c/z}$ | $20.32 \pm 0.74^{c/z}$ | $3.25 \pm 0.10^{c/y}$ |
| G+BCG | 2 | $91.53 \pm 0.46^{a/w}$ | $-5.73 \pm 0.02^{a/w}$ | $31.57 \pm 0.84^{a/z}$ | $6.18 \pm 0.13^{a/w}$ |
| | 6 | $57.41 \pm 0.23^{b/w}$ | $-10.66 \pm 1.13^{b/w}$ | $-32.33 \pm 1.89^{b/w}$ | $12.94 \pm 0.50^{b/z}$ |
| | 11 | $43.33 \pm 1.24^{c/w}$ | $-5.21 \pm 0.42^{c/w}$ | $-47.01 \pm 0.48^{c/w}$ | $14.63 \pm 0.09^{c/z}$ |

Reported values for each gelatin film are means \pm standard deviation ($n=9$). Different letters (a, b, c, d) in the same column indicate significant differences ($p<0.05$) among the different acid-base indicators for the same pH of film-forming dispersion, according to Tukey's test. Different letters (w, x, y, z) in the same column indicate significant differences ($p<0.05$) among the different pH of film-forming dispersion for the same film formulation, according to Tukey's test.

Table 3. Thickness, moisture content (MC), water solubility (WS), water vapor permeability (WVP) and glass transition temperature (Tg) of gelatin-based films (G) added or not with methyl orange (MO), neutral red (NR), and bromocresol green (BCG) at pH 2, 6 and 11.

| Film | pH | Thickness (μm) | MC (%) | WS (%) | WVP * 10^{11} ($\text{gH}_2\text{O/s.m.Pa}$) | Tg ($^{\circ}\text{C}$) |
|-------|----|--------------------------------|-------------------------------|-------------------------------|---|-------------------------------|
| G | 2 | 49.5 \pm 3.9 ^{a/x} | 19.2 \pm 0.5 ^{a/x} | 49.6 \pm 1.6 ^{a/x} | 7.63 \pm 0.84 ^{a/x} | -7.9 \pm 0.7 ^{a/x} |
| | 6 | 51.0 \pm 3.0 ^{a/x} | 22.1 \pm 0.6 ^{a/y} | 37.6 \pm 2.7 ^{a/y} | 6.54 \pm 0.34 ^{a/x} | -6.3 \pm 2.0 ^{a/x} |
| | 11 | 47.8 \pm 3.4 ^{a/x} | 21.5 \pm 0.3 ^{a/y} | 37.6 \pm 1.8 ^{a/y} | 7.96 \pm 0.36 ^{ab/x} | -6.8 \pm 0.7 ^{a/x} |
| G+MO | 2 | 45.4 \pm 2.2 ^{a/x} | 16.9 \pm 0.8 ^{b/x} | 30.7 \pm 2.9 ^{b/x} | 8.28 \pm 0.86 ^{a/x} | -7.3 \pm 1.0 ^{a/x} |
| | 6 | 48.1 \pm 2.3 ^{a/x} | 16.3 \pm 0.5 ^{b/x} | 23.2 \pm 1.4 ^{b/y} | 7.00 \pm 0.96 ^{a/x} | -5.1 \pm 0.5 ^{b/x} |
| | 11 | 51.1 \pm 3.0 ^{a/x} | 17.3 \pm 0.3 ^{b/x} | 15.2 \pm 0.1 ^{b/z} | 6.71 \pm 0.19 ^{a/x} | -6.9 \pm 0.5 ^{a/x} |
| G+NR | 2 | 49.7 \pm 3.3 ^{a/x} | 17.4 \pm 0.6 ^{b/x} | 53.5 \pm 4.4 ^{a/x} | 8.90 \pm 0.94 ^{a/x} | -7.7 \pm 0.5 ^{a/x} |
| | 6 | 50.2 \pm 1.8 ^{a/x} | 17.5 \pm 1.1 ^{b/x} | 38.1 \pm 1.9 ^{a/y} | 8.83 \pm 0.75 ^{a/x} | -5.7 \pm 1.2 ^{a/x} |
| | 11 | 50.7 \pm 1.9 ^{a/x} | 16.5 \pm 0.1 ^{b/x} | 34.1 \pm 1.0 ^{a/y} | 8.58 \pm 0.78 ^{bc/x} | -6.7 \pm 0.1 ^{a/x} |
| G+BCG | 2 | 46.0 \pm 2.1 ^{a/x} | 20.5 \pm 0.7 ^{a/x} | 51.3 \pm 0.9 ^{a/x} | 9.12 \pm 0.13 ^{a/xy} | -6.2 \pm 1.1 ^{a/x} |
| | 6 | 49.5 \pm 3.7 ^{a/x} | 23.3 \pm 1.2 ^{a/y} | 21.5 \pm 0.5 ^{b/y} | 8.62 \pm 0.25 ^{a/x} | -4.2 \pm 0.5 ^{b/x} |
| | 11 | 49.4 \pm 6.5 ^{a/x} | 20.3 \pm 0.2 ^{a/x} | 49.8 \pm 5.6 ^{c/x} | 9.90 \pm 0.70 ^{c/y} | -6.5 \pm 1.1 ^{a/x} |

Reported values for each gelatin film are means \pm standard deviation ($n=9$ for thickness; $n=3$ for MC, WS and WVP; $n=2$ for Tg). Different letters (a, b, c) in the same column indicate significant differences ($p<0.05$) among the different acid-base indicators for the same pH of film-forming dispersion, according to Tukey's test. Different letters (x, y, z) in the same column indicate significant differences ($p<0.05$) among the different pH of film-forming dispersion for the same film formulation, according to Tukey's test.

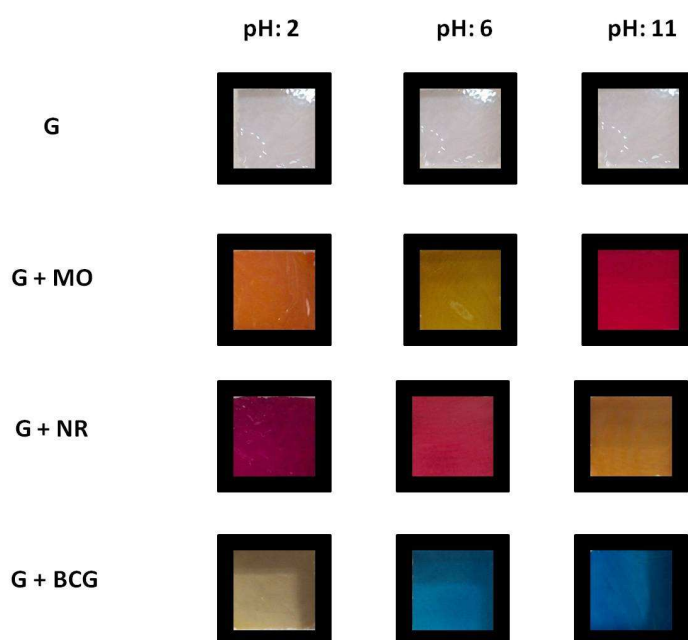


Figure 1. Appearance of control gelatin-based films (G) and those added with methyl orange (G+MO), neutral red (G+NR), and bromocresol green (G+BCG) at pH 2, 6 and 11.

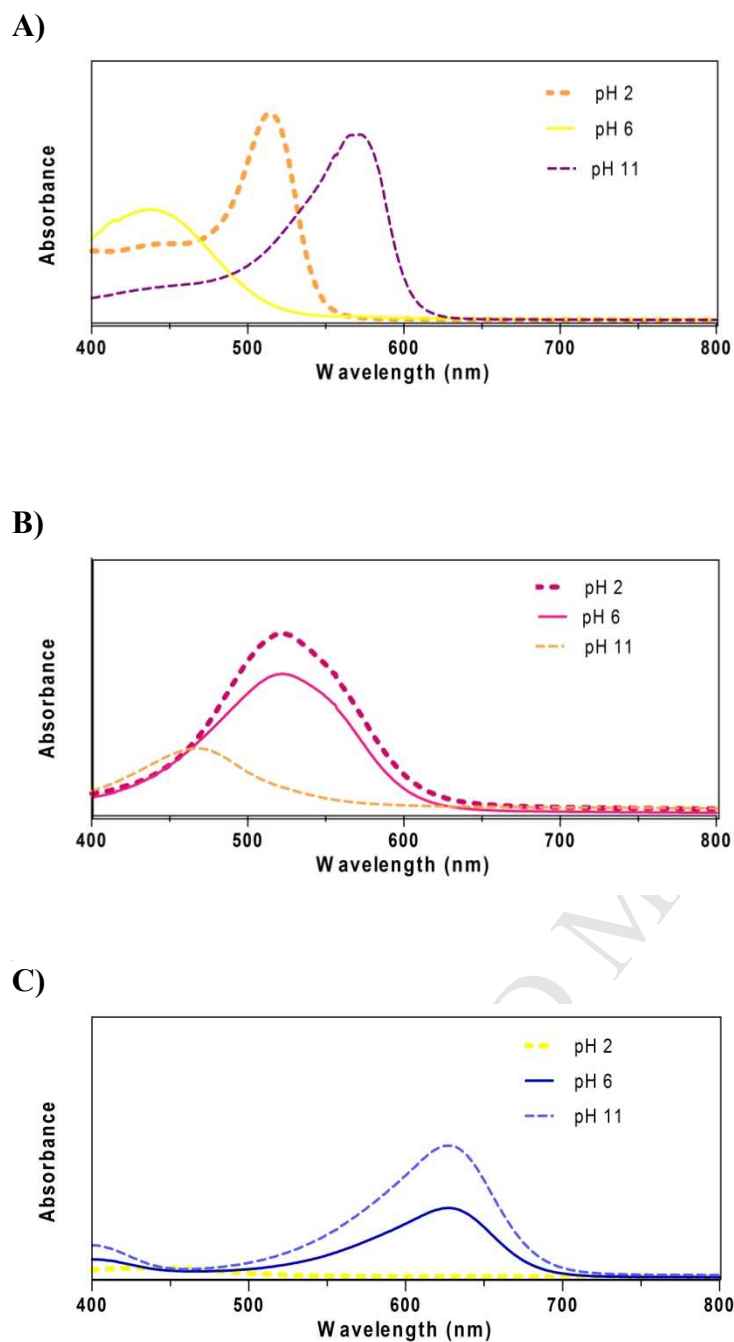
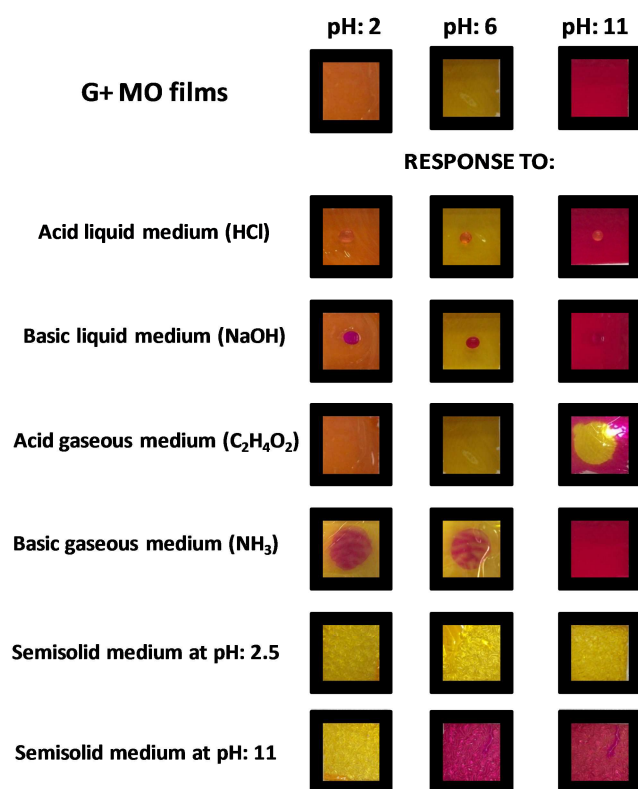
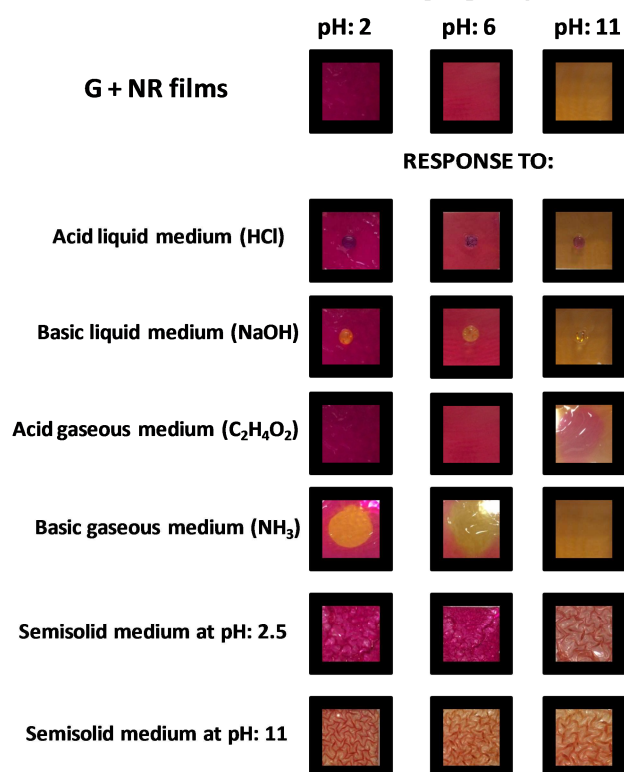


Figure 2. Visible absorption spectra (400-800 nm) of gelatin-based films added with methyl orange (A), neutral red (B) and bromocresol green (C) at pH 2, 6, and 11 respectively.

A)



B)



C)

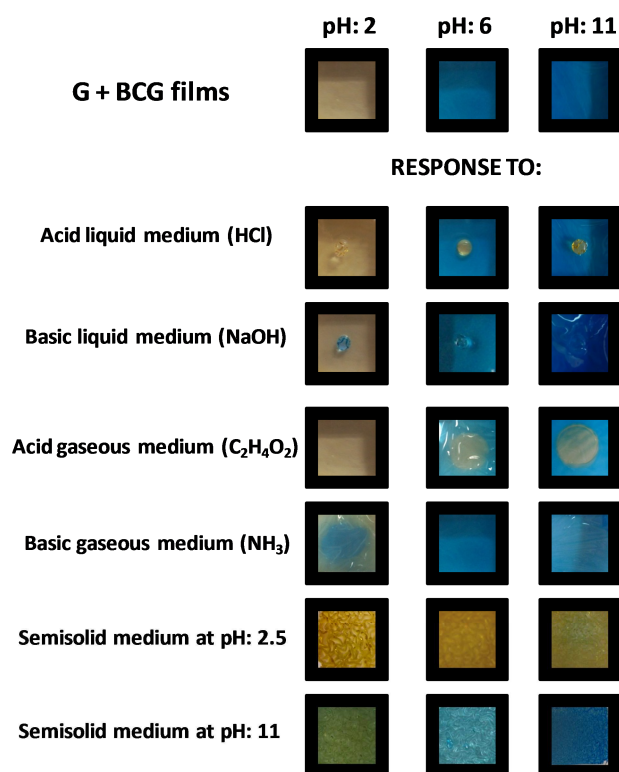
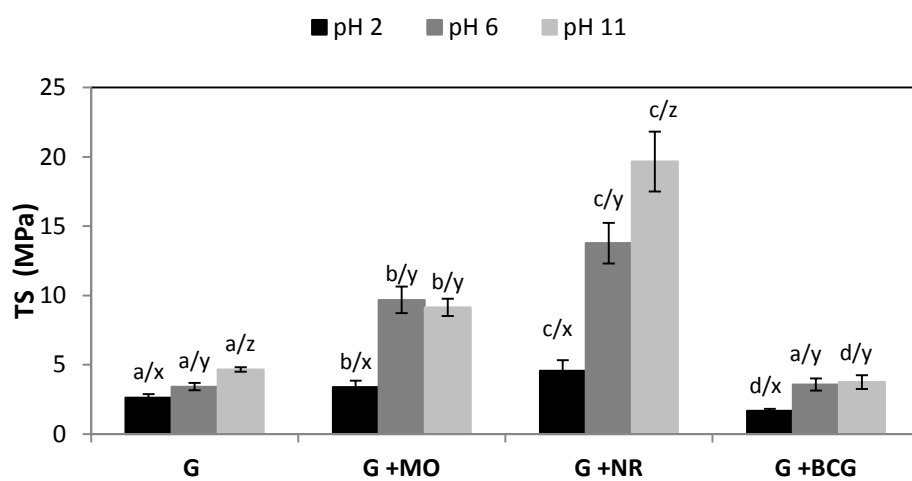
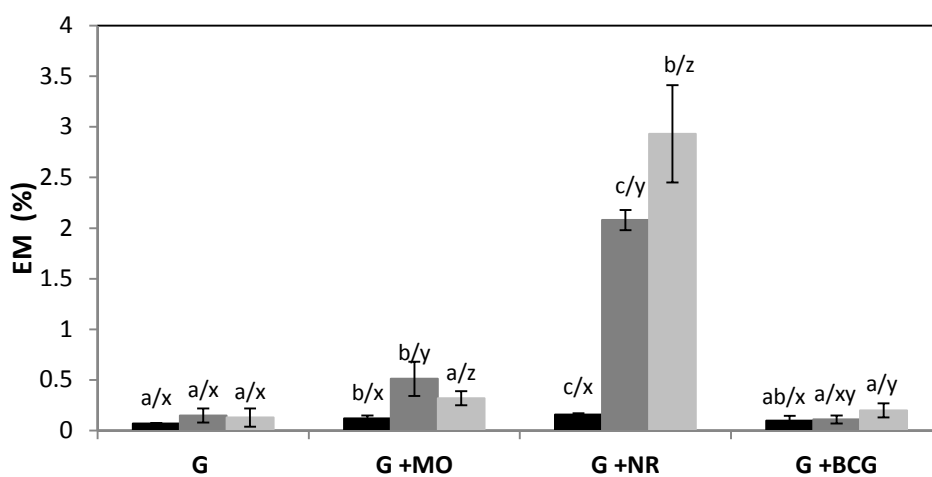


Figure 3. Response of gelatin-based films added with methyl orange (G+MO, **A**), neutral red (G+NR, **B**), and bromocresol green (G+BCG, **C**) at pH 2, 6 and 11 after being in contact with liquid, gaseous and semisolid media of different pHs.

A)



B)



C)

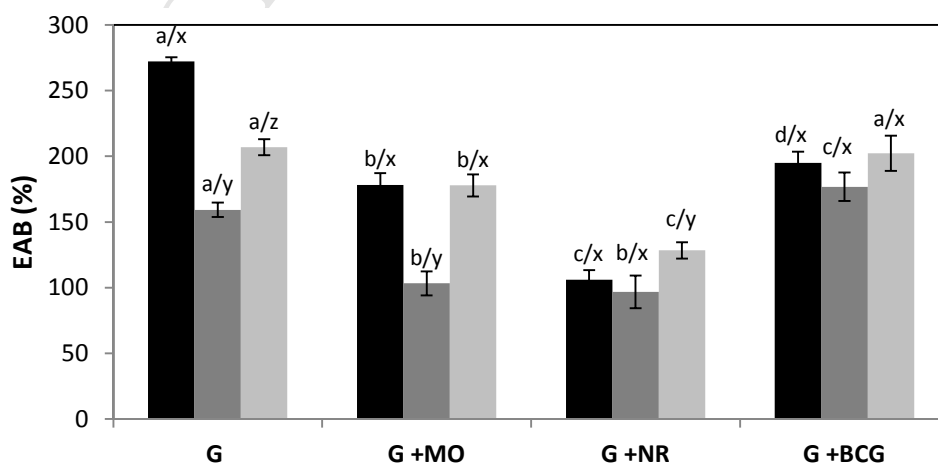


Figure 4. Mechanical properties of gelatin-based films obtained at different pH (2, 6 and 11) added or not with different acid-base indicators (MO, NR, and BCG). **A)** Tensile strength (TS). **B)** Young's modulus (EM). **C)** Elongation at break (EAB).

Reported values for each gelatin film are means \pm standard deviation ($n=12$). Different letters (a, b, c, d) indicate significant differences ($p<0.05$) among the different acid-base indicators for the same pH of film-forming dispersion, according to Tukey's test. Different letters (x, y, z) indicate significant differences ($p<0.05$) among the different pH of film-forming dispersion for the same film formulation, according to Tukey's test.

Highlights

- Smart gelatin films added with synthetic acid-base indicators were developed
- Films modified their color after being in contact with media at different pHs
- Films' response was evaluated against gaseous, liquid and semisolid media
- Protein matrix didn't interfere with the discoloration of the acid-base indicators
- Acid-base indicator's presence affected the physicochemical properties of films