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1 **Conception of novel blue crab chitosan films crosslinked with**
2 **different saccharides via the Maillard reaction with improved**
3 **functional and biological properties.**

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20 **Abstract**

21 This work aimed to modify blue crab chitosan-based films through the Maillard reaction
22 (MR) as a novel alternative to improve their functional and biological properties. To this end,
23 different saccharides (glucose (aldohexose), fructose (ketohehexose), xylose (aldopentose) and
24 arabinose (aldopentose)), at different weight ratios 0.5, 1.0 and 2.0% (g/100 g polymer), were
25 studied, and films were heated at 90 °C for 24 h. Based on color changes and browning index
26 measurements, the extent of MR was the highest with aldopentoses, whereas hexoses and
27 particularly ketohehexoses, exhibited a relative crosslinking rate. These findings were further
28 reflected with an improvement in treated films mechanical properties and thermal degradation
29 temperatures, and advantageously, barrier properties against UV light and water. In addition,
30 the MR-modified Cs-based films antioxidant activity was interestingly enhanced with mainly
31 aldopentoses. Consequently, MR crosslinked chitosan-based films are promising alternative for
32 active and functional packaging able of food oxidation hindering, especially using
33 aldopentoses.

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39 **Keywords:** Chitosan; Films; Crosslinking; Maillard reaction; *In vitro* characterization.

40 **1. Introduction**

41 Nowadays, there has been several concerns towards the alarming natural resources
42 lessening, health and environmental hitches of petroleum-based synthetic polymers,
43 traditionally and massively used in medical and food industries (Al Jahwari and Pervez, 2019;
44 Liu *et al.*, 2019; Sponchioni, Palmiero and Moscatelli, 2019). Therefore, intensive researches,
45 in the field of food protection, has focused on the conception of a natural and non-toxic coating
46 material, able of effectively enhancing the shelf-life of fresh or packaged commercialized food,
47 as alternative to synthetic films (Assadpour and Jafari, 2019; Galiano *et al.*, 2018).

48 These bio-based packaging should be able to perfectly hinder the UV radiation, prevent
49 oxidative damages and encounter food-borne pathogens developpement, for the protection of
50 food and extending their shelf life, without ideally loss of their mechanical integrity and
51 behavior characteristics (Vilela *et al.*, 2017). Among most studied biopolymers, chitosan is an
52 inspiring and propitious polymer, of a polysaccharide nature, competently applied as an
53 alternative in agriculture, food and pharmaceutical industries (Vukajlovic, Parker, Bretcanu and
54 Novakovic, 2019; Shariatinia, 2018). Although the applicability of chitosan-based biomaterials
55 is knowing a noteworthy increase (Zhai, Bai, Zhu, Wang and Luo, 2018), still certain
56 physicochemical properties of the chitosan-based films are deficient and require, thereby, to be
57 amended in order to feat industrial needs, such as light and water barrier properties, since
58 packaging materials' water content and light (UV specially) transmission basically affected
59 packaged or coated materials' shelf life), as well mechanical resistance (Akyuz *et al.*, 2017). In
60 fact, regarding materials applied or expected for food packaging, mechanical properties
61 evaluation is mandatory for shaping their performance to sustain several sorts of stresses
62 occurring during packaging applications processes.

63 Several types of treatments can be used for the modification of edible films, in order to
64 improve their structural properties (such as sensitivity to water), barrier characteristics (light

65 barrier and water vapor or gas permeability), biological properties (i.e. antioxidant and
66 antimicrobial activities), etc., including the combination of two polymers, physical treatments
67 such as heat treatment and irradiation and biochemical treatments such as the Maillard reaction
68 (MR) (Mujtaba *et al.*, 2019; Kamboj, Singh, Tiwary and Rana, 2015).

69 Given the good antioxidant and antibacterial activities of MR products in food industry,
70 their use as a preservative is attracting increasing interest (Sung, Chang, Chou and Hsiao, 2018;
71 Cai *et al.*, 2016; Gullon *et al.*, 2016; Wu *et al.*, 2014). The MR, also called non-enzymatic
72 browning, is a chemical reaction of browning of amino acids in the presence of sugars
73 (Umemura and Kawai, 2007). MR is the main reaction responsible for the transformation of
74 precursors into dyes and flavoring compounds during food processing and preservation (Xu,
75 Huang, Xu, Liu and Xiao, 2019), which can be influenced by many factors, including reagent
76 concentration, temperature, heating time, initial pH, and reagent characteristics.

77 In a previous work (Hamdi, Nasri, Li and Nasri, 2019a), the improvement of the barrier,
78 mechanical and biological properties of blue crab chitosan-based films was envisaged, by
79 incorporation of carotenoproteins used both as polymers and antioxidant and antimicrobial
80 agents. The addition of carotenoproteins at 15% (w/w Cs) in the blue crab chitosan film-forming
81 matrix allowed an improvement in the barrier, color, thermal and biological (antioxidant and
82 antimicrobial) properties. However, a reduction in their mechanical resistance was noted. Thus,
83 the present work is interested in a novel alternative to improve the physical and biological
84 properties of blue crab chitosan-based films, by complex crosslinking coupled with a heat
85 treatment. Indeed, recently, researches regarding modifying proteins properties through the MR
86 revealed that protein-saccharide grafts are advantageous as an innovative functional biopolymer
87 with excellent emulsifying, antioxidant and antimicrobial properties for food and
88 pharmaceutical applications (Kchaou *et al.*, 2018).

89 Although many studies, dealing with chitosan-based films (Fernandez-de Castro *et al.*,
90 2016; Rubentheren *et al.*, 2016; Leceta, Guerrero, Ibarburu, Dueñas and de la Caba, 2013) and
91 chitosan-MR (Wang, Liu, Liang, Yuan and Gao, 2014; Lakshmi Kosaraju, Weerakkody and
92 Augustin, 2010; Umemura and Kawai, 2008), have been performed, and the factors affecting
93 the reaction, including sugar concentration, temperature and reaction time have been evaluated
94 (Gullon *et al.*, 2016), there are no many previous reports on the effect of saccharides
95 type/chemical structure on the physicochemical and antioxidant properties of films based on
96 chitosans and different types of saccharides. Therefore, in the present work, we have chosen to
97 compare the reactivity of chitosan with different mono-saccharides, glucose (aldohexose),
98 fructose (ketohehexose), xylose (aldopentose) and arabinose (aldopentose), at different weight
99 ratios 0.5, 1.0 and 2.0% (g/100 g polymer).

100 **2. Materials and methods**

101 **2.1. Materials**

102 D-anhydrous-glucose ($C_6H_{12}O_6$; 180 g mol⁻¹), D-anhydrous fructose ($C_6H_{12}O_6$; 180 g
103 mol⁻¹), L-anhydrous-arabinose ($C_5H_{10}O_5$; 150 g mol⁻¹) and L-anhydrous-xylose ($C_5H_{10}O_5$; 150
104 g mol⁻¹), purchased from Sigma-Aldrich (France), were applied as reducing sugar for the
105 Maillard reaction initiation in Cs-based films. In the present work, the other used chemical
106 reagents from commercial sources were of analytical grade and employed without further
107 purifications.

108 **2.2. Extraction of blue crab chitosan**

109 Chitosan (Cs), with acetylation degree of 8%, as characterized by ¹³C NMR, molecular
110 weight Mw of 115 kDa and intrinsic viscosity [η] of 3432 ml/g, based on the size exclusion
111 chromatography, was obtained from blue crab shells chitin, through N-deacetylation with NaOH

112 12.5 M, at a ratio of 1/10 (w/v), for 4 h at 140 °C, as reported in our previous study (Hamdi *et*
113 *al.*, 2018).

114 **2.3. Preparation of Cs-based MR-treated films**

115 Film-forming solutions were prepared by dissolving Cs at a concentration of 2% (w/v) in
116 aqueous solution of acetic acid 0.15 M. Then, sugars (glucose, fructose, arabinose and xylose)
117 were added under gentle stirring at different concentrations (0.5, 1.0 and 2.0%; w/w dry Cs
118 matter). Glycerol was added as a plasticizer to the film-forming solutions at a concentration of
119 15% (w/w dry Cs matter). After stirring for 24 h at 25 °C and degassing, film-forming solutions
120 were left standing for 12 h at 25 °C and centrifuged for 10 min at 8000 g. Thereafter, films were
121 prepared by casting film-forming solutions (25 ml) on Petri dishes (13.5 cm diameter) and were
122 left to dry for 48 h at 25 °C, until complete solvent evaporation.

123 The well-dried films were peeled from the plate and heated for 24 h in an oven at a
124 temperature of 90 ± 2 °C and an average relative humidity (RH) value of approximately 30%,
125 to apply the heat-treatment for MR induction. Non-heated films are considered as controls and
126 saccharide-free chitosan film is considered as blank. Finally, all designed films were maintained
127 at 25 °C at a RH of 50%, for subsequent characterizations.

128 **2.4. Analytical methods**

129 **2.4.1. Films thickness**

130 The thickness of prepared films was measured by using a micrometer (Digimatic IP65,
131 Mitutoyo, France), reporting the average value of 5-6 measurements taken at random locations.
132 Thickness results were taken into account for mechanical properties.

133 **2.4.2. Films moisture content**

134 Water content (WC) of prepared films was performed in an oven at 105 °C, where
135 samples (approximately 100 mg) were dried until constant weight was reached (sample dry

136 weight). The moisture content of films was determined by measuring the mass loss of each film
137 in triplicate and expressed as follows [21]:

$$138 \quad \text{WC (\%)} = \frac{M_0 - M_1}{M_0} \times 100 \quad \text{Eq (1)}$$

139 where M_0 and M_1 are respective weights (g) of films before and after drying.

140 **2.4.3. Films water solubility**

141 Films solubility in water was determined in triplicate based on the method reported by
142 Gennadios, Handa, Froning, Weller and Hanna (1998). Film samples (2 cm x 5 cm; about 100
143 mg), previously weighed, were immersed in 30 ml distilled water containing 0.1% (w/v)
144 antimicrobial agent (sodium azide), for 24 h at 25 °C. Afterwards, tubes containing film samples
145 were centrifuged at 3000 g, 25 °C for 10 min. Supernatants were removed, and solubilized film
146 portion was estimated by drying the obtained undissolved debris, at 105 °C for 24 h. Water
147 solubility (WS) was determined according the following equation:

$$148 \quad \text{WS (\%)} = \frac{[M_i \times (100 - \text{WC}) - M_f]}{M_i \times (100 - \text{WC})} \times 100 \quad \text{Eq (2)}$$

149 where M_i and M_f are initial and final respective masses (g) of films and WC is water content of
150 each film sample (%).

151 **2.4.4. Films water contact angle**

152 A goniometer (Drop Shape Analyzer 30 from Kruss GmbH), equipped with an image
153 analysis software (ADVANCE) was used for the study of the films water contact angle (WCA),
154 considering the sessile drop method. Briefly, a droplet of water (approximately 2 ml) was
155 dropped on the film surface using a precision syringe, in an environmental chamber with a
156 constant environment at a temperature of 25 ± 2 °C and a RH of 50%. WCA, expressed as °,

157 was measured on both sides of the drop and the average of five measurements was determined
158 and reported.

159 **2.4.5. Films water vapor permeability**

160 The gravimetric method, adapted by Debeaufort, Martin-Polo and Voilley (1993) for
161 hydrophilic polymers, was applied for the determination of films water vapor permeability
162 (WVP). First, to permit films equilibration, samples were conditioned at 25 °C and 50% RH for
163 a minimum equilibration time of 48 h. Films thickness measurements values and statistical error
164 were considered in the WVP measurements. WVP calculations were based on maintaining film
165 samples between two Teflon rings at the glass cell upper. The WVP cell containing KCl saline
166 solution (100% RH), was placed into a climatic chamber (KBF 240 Binder, ODIL, France)
167 maintained at 25 °C and a RH of 30%. Once the equilibrium state was reached, the value of
168 WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was calculated, based on the change in the absolute value of the weight
169 loss as a function of time, as follows:

$$170 \quad \text{WVP (g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}) = \frac{\Delta m}{\Delta t} \times \frac{1}{A} \times \frac{1}{\Delta p} \times e \quad \text{Eq (3)}$$

171 where $\Delta m/\Delta t$ is the weight of moisture loss per unit of time (g/s), A is the film area exposed to
172 the transfer ($1.39 \times 10^4 \text{ m}^2$), e is the film thickness (m), and Δp is the water vapor partial pressure
173 differential between the two sides of the film (Pa).

174 **2.4.6. Optical properties of films**

175 **2.4.6.1. Color features**

176 Films color parameters were measured, using a portable colorimeter (Minolta Chroma
177 Meter CR-300, CIE, 1976) and the color was recorded using the color parameters CIE L* a*
178 b*. L* indicates transparency and its value varies from 0 (black) to 100 (white), a* is a measure
179 of green (-a*) / redness (+ a*) and b* is the measure of blue (-b*) / yellow (+ b*). The

180 colorimeter was calibrated against a standard Minolta standard reflector plate before each actual
 181 color measurement. In addition, for the derived color parameters, the total color difference (ΔE),
 182 of prepared films were calculated using the following equations:

$$183 \quad \Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \mathbf{Eq (4)}$$

184 where L_0^* , a_0^* , b_0^* are the colorimetric parameters of the standard are ($L^* = 97.25$, $a^* = -0.2$
 185 and $b^* = 2.25$) and L^* , a^* , b^* are the values for the films obtained. The C axis represents
 186 Chroma (or saturation). It ranges from 0, which is completely unsaturated (neutral gray, black
 187 or white) to 100 for very high Chroma (saturation) or purity of color.

188 **2.4.6.2. Maillard-treated films browning index**

189 Films browning index (BI) was determined, as reported by Matiacevich and Pilar Buera
 190 (2006), based on the CIE L^* a^* b^* values, using the following equation:

$$191 \quad BI = \frac{100 (z - 0.31)}{0.172} \quad \mathbf{Eq (5)}$$

192 where,

$$193 \quad z = \frac{a + 1.75 (L)}{5.645 (L) + a - 3.012 (b)} \quad \mathbf{Eq (6)}$$

194 **2.4.6.3. Films UV barrier and transparency properties**

195 The transmittance spectra (200-800 nm) of the developed films were made using a UV-
 196 Visible spectrophotometer (T70, UV/vis spectrometer, PG Instruments Ltd., China). Thus, the
 197 films are cut to obtain rectangles of 1 cm x 3 cm and directly placed in the test cell of the
 198 spectrophotometer. Air was used as a reference. This measurement aims to evaluate the barrier
 199 effect of UV films and to determine their transparency, considering the following equation:

$$200 \quad \text{Transparency (\%)} = \frac{-\log T_{600}}{e} \quad \mathbf{Eq (7)}$$

201 where T_{600} is the fractional transmittance at 600 nm and e is the thickness (mm) of the films.
202 The lower transparency of the film is reflected by a greater transparency value.

203 **2.4.7. Infrared spectroscopy analysis**

204 The FT-IR spectra of the prepared were performed using a spectrometer (Agilent
205 Technologies, Carry 630 series) equipped with an attenuated reflection accessory (ATR)
206 containing a diamond/ZnSe crystal, at room temperature (25 °C) in the spectral range
207 frequencies of 650-4000 cm^{-1} . For each spectrum, 32 scans of interferograms were averaged
208 and the spectral resolution was 4 cm^{-1} . Data analysis and treatment was carried out by using the
209 OMNIC Spectra software (ThermoFisher Scientific).

210 **2.4.8. Films thermal properties**

211 ***2.4.8.1. Differential Scanning Calorimeter analysis***

212 Differential Scanning Calorimeter (Modulated DSC Q20, TA Instruments), equipped
213 with a liquid nitrogen cooling system, was used to investigate the thermal properties of prepared
214 films, allowing the estimation of melting and crystallization point, as well as the glass transition
215 of the macromolecular materials. Film samples were accurately weighed into aluminum pans
216 and sealed. An empty capsule served as an inert reference and the apparatus was calibrated
217 using indium. The thermal profile of chitosan samples was analyzed in a temperature range of
218 0-225 °C, at a heating rate scan of 10 °C/min, under nitrogen flow rate of 50 ml/min.
219 Thermograms were then analyzed by using TA Universal V4.5A software.

220 ***2.4.8.2. Thermogravimetric analysis***

221 Thermogravimetric analysis (TGA Q500 High Resolution, TA Instruments), operating
222 under nitrogen flow, was used to study the thermal stability of Cs-based MR-treated films. The
223 progressive change in mass (%) as a function of temperature, is recorded. Thus, films
224 (approximately 4 mg) were heated from 25 to 700 °C at a heating rate of 20 °C/min and

225 constantly measured with an accuracy of 0.01 mg. Cs-based films thermograms were
226 subsequently recorded, analyzed and treated using the TA Universal V4.5A software (TA
227 Instruments, Waters, New Castle, UK).

228 **2.4.9. Films mechanical properties**

229 Films mechanical properties were investigated based on the determination of the tensile
230 strength (TS, MPa) and elongation at break (EAB, %) parameters. A rheometer apparatus
231 (Physica MCR, Anton Paar, GmbH, France) equipped with mechanical properties measuring
232 geometry was used. To this end, films rectangular samples (1.0 cm x 4.5 cm) were cut with an
233 accurate width and parallel sides throughout the entire length.

234 Prior to analysis, all the films samples were equilibrated at 25 °C and 50% RH for two
235 weeks and their thickness were measured. Based on the ISO standard, equilibrated films
236 samples, retained in the extension grips of the measuring system, were subjected to a uniaxial
237 tensile test, with a deformation rate of 5 mm/min until breaking. Rheoplus software was used
238 for the estimation of TS and EAB, corresponding respectively to the maximum load and the
239 final extension at break from the stress-strain curves. Average values from at least six
240 measurements were reported.

241 **2.4.10. Films microstructure**

242 The cross-section of films was determined using scanning electron microscopy (SEM)
243 (Hitachi S4800), at an angle of 90 ° to the surface. Prior to imaging the film cross-section, film
244 samples were cryofractured by immersion in liquid nitrogen, cut and fixed on the SEM support
245 using double side adhesive tape, under an accelerating voltage of 2.0 kV and an absolute
246 pressure of 60 Pa, after sputter coating with a 5 nm thick gold.

247 **2.5. Films antioxidant potential**

248 **2.5.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals scavenging ability**

249 The ability of films (10 mg) to scavenge DPPH radical was determined according to the
250 method of Bersuder, Hole and Smith (1998). The absorbance change, at 517 nm between the
251 control and the sample, was used to calculate the scavenging activity. DPPH radical-scavenging
252 activity of Cs-based MR-treated films was evaluated and computed considering the following
253 equation:

$$254 \quad \text{DPPH Radical scavenging activity (\%)} = \frac{A_C + A_B - A_R}{A_C} \times 100 \quad \text{Eq (8)}$$

255 where A_C is the absorbance of the control reaction, A_R and A_B were the absorbances of Cs-
256 based films in the reaction mixture and without addition of DPPH solution, respectively. Results
257 were expressed as means of experiments performed in duplicate.

258 **2.5.2. Reducing power activity assay**

259 The ability of films (10 mg) to reduce iron (III) was determined according to the method
260 of Yildirim, Mavi and Kara (2001). The absorbance of the resulting solutions was measured at
261 700 nm, after incubation at room temperature for 10 min. Higher absorbance of the reaction
262 mixture indicated higher reducing power. The test was carried out in duplicate and BHA was
263 used as a standard.

264 **2.5.3. Metal-chelating activity**

265 The chelating activity of films (10 mg) towards ferrous ion (Fe^{2+}) was studied as reported
266 by Decker and Welch (1990). EDTA was used as reference and the chelating activity (%) was
267 computed using the following equation:

$$268 \quad \text{Chelating activity (\%)} = \frac{A_C + A_B - A_R}{A_C} \times 100 \quad \text{Eq (9)}$$

269 where A_C is the absorbance of the control reaction, A_R and A_B were the absorbance of
270 Cs-based films in the reaction mixture and without addition of Ferrozine solution, respectively.
271 Results were expressed as means of experiments performed in duplicate.

272 **2.6. Statistical analysis**

273 All experiments were carried out in duplicate, excepting films mechanical properties,
274 where analysis was repeated at least six times, and average values with standard deviation errors
275 were reported. Mean separation and significance were analyzed using the SPSS software
276 package ver. 17.0 professional edition (SPSS, Inc., Chicago, IL, USA) using ANOVA analysis.
277 Differences between the different Cs-based films crosslinked with the different saccharides and
278 heated or not at 90 °C for 24 h, were considered significant at $p < 0.05$.

279 **3. Results and Discussion**

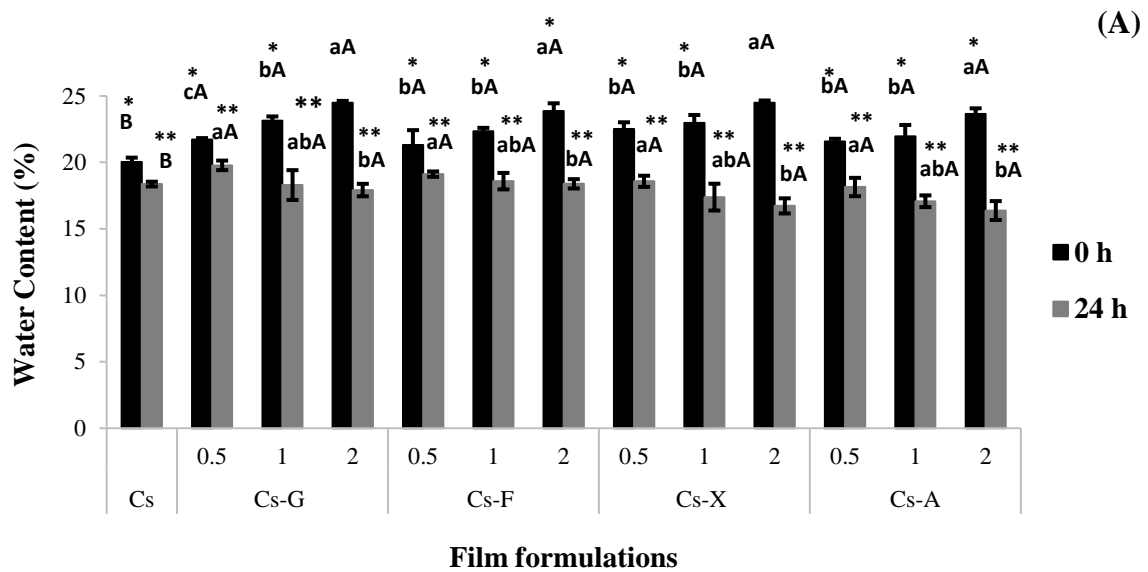
280 **3.1. Water resistance and barrier features of Cs-based films as influenced by the MR**

281 **3.1.1. Water content measurement**

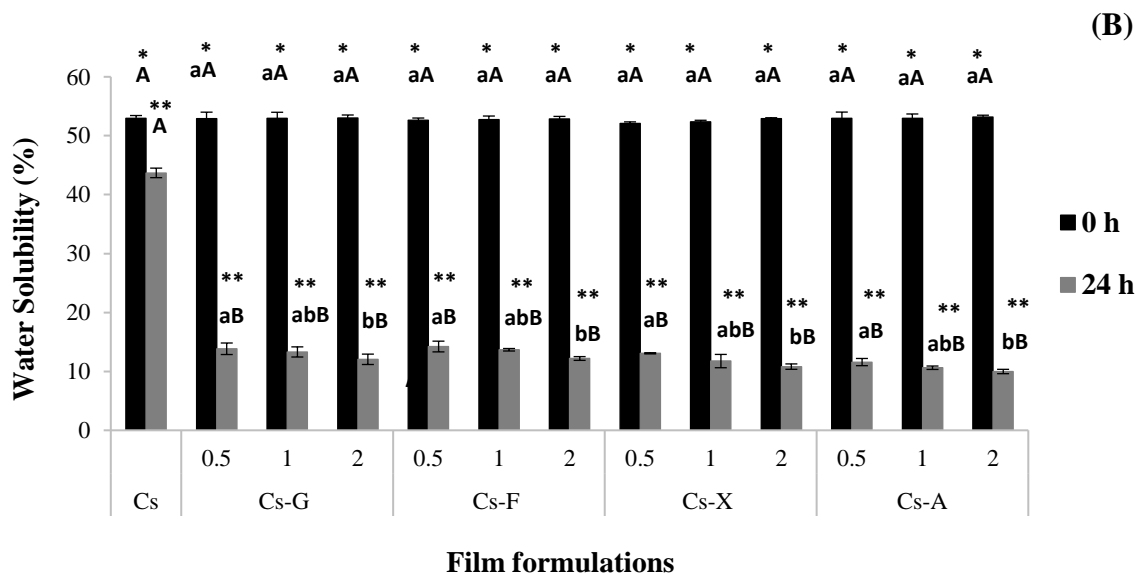
282 Packaged or coated materials' shelf life is affected by packaging materials' WC (Kchaou
283 *et al.*, 2018). WC values (**Fig. 1A**) reveal that, for non-heated Cs-based films, humidity values
284 increased from 20% for blank (non-added) Cs-based film to 24% for 2.0% (w/w Cs) crosslinked
285 Cs-saccharides films ($p < 0.05$), independently of the added saccharide (aldose or ketose, pentose
286 or hexose). However, at concentrations of 0.5 and 1.0% (w/w Cs), no significant differences
287 were noted ($p > 0.05$). The very well-known hygroscopicity of saccharides could explain the
288 increase of WC of the non-heated Cs-saccharides films (Hazaveh, Mohammadi Nafchi and
289 Abbaspour, 2015).

290 After induction of the MR (heat treatment at 90 °C for 24 h), WC decreased gradually
291 with the increase of saccharide content from 0.5% (w/w Cs) to 2.0% (w/w Cs), depending on
292 the type of the saccharide used ($p < 0.05$), due to the interaction between the amino groups of Cs
293 and the carbonyl groups of the tested saccharide, resulting in polar groups decrease and the
294 disruption of Cs-water interactions due to hampering of the hydroxyl and amino groups
295 availability (Rui *et al.*, 2017). In fact, regarding the Cs-arabinose crosslinked films, the WC
296 dropped to 18.15%, 17.08% and 16.38% at 0.5, 1.0 and 2.0% (w/w Cs), which could be

297 explained by free water loss from MR-treated films after being heated at 90 °C. Moreover, the
 298 following order of reactivity could be observed: fructose < glucose < xylose < arabinose. For
 299 example, at a concentration of 2.0% (w/w Cs), after heat treatment, the WC values reached
 300 16.38%, 16.73%, 17.92% and 18.39%, for Cs-arabinose, Cs-xylose, Cs-glucose and Cs-fructose
 301 crosslinked films, respectively, vs. 18.37% for blank Cs-based film.



302



303

304 **Figure 1:** Water content (WC%) (A) and water solubility (WS%) (B) of Cs-based films
 305 conjugated with different saccharides, at different mass ratios (0.5, 1.0 and 2.0 %; w/w Cs),
 306 through MR at 90 °C as a function of time (0 and 24 h). Cs: Chitosan, A: Arabinose, F: Fructose, G:
 307 Glucose, X: Xylose. Different letters (a-c) in the same films group are significantly different as determined by
 308 ANOVA test (p<0.05). Different letters (A-B) indicated significant differences between different films groups at
 309 the same heating time (p<0.05). Different symbols (*,**) indicated significant differences between heated and non-
 310 heated films for the same group (p<0.05).

311 This finding suggested that the extent of the MR, as reflected by films water content
312 decrease, was influenced by the type of saccharides, where pentoses (arabinose and xylose),
313 with less carbons in their macromolecular chain, showed more reactivity, compared to the
314 hexoses (fructose and glucose), with more carbons (Gullon *et al.*, 2016). Particularly, aldoses
315 (arabinose, xylose and glucose), with a carbonyl group located at one end of the carbon chain,
316 were more reactive than ketoses (fructose), with a carbonyl group situated inside the carbon
317 chain, resulting in the lowest WC values after crosslinking with aldopentoses, proving higher
318 covalent crosslinking extent and lower polar groups availability.

319 **3.1.2. Study of water solubility**

320 Films WS, related with the biomaterial hydrophobicity/hydrophilicity, is a crucial feature
321 defining biopolymeric-based films applications. WS results, shown in **Fig. 1B**, highlight that
322 blank Cs-based film was sensitive to water, exhibiting WS of 52%. For the non-heated Cs-
323 saccharides films, WS values were maintained constant, independently of the type and dose of
324 added saccharide, compared to the blank Cs-based film ($p>0.05$). However, as expected, due to
325 the crosslinking, films WS decreased significantly with the heat treatment for the different Cs-
326 saccharide ratios ($p<0.05$). For example, after 24 h MR time, WS reached values of 11.58%,
327 10.62% and 9.98% for Cs-arabinose mass ratios of 0.5, 1.0 and 2.0% (w/w Cs), respectively,
328 vs. 43.69 for blank Cs-based film heated at 90 °C for 24 h. Compared to blank film, when added
329 to the Cs film-forming solution, overall tested saccharides acted as crosslinking agent,
330 permitting the creation of covalent bonds with the Cs polymeric matrix, and thereby, the
331 decrease of WS values (Kamboj *et al.*, 2015). In fact, the principal mechanisms of MR are the
332 formation of the initial products, called Schiff bases, which form Amadori products via
333 rearrangement. These latter undergo further reactions to form irreversible advanced glycation
334 end products, called melanoidins (Kchaou *et al.*, 2018).

335 Moreover, WS decrease was saccharide dose dependent. In fact, WS of films with higher
336 saccharide contents (2.0%, w/w Cs), and thereby, higher crosslinking rate, decreased more
337 rapidly, compared to films with relative crosslinking degree, as a result of lower saccharide
338 content (0.5%, w/w Cs).

339 As predicted, MR was more facilitated with pentoses and aldoses than hexoses and
340 ketoses, knowing that higher crosslinking resulted in lower the solubility. For 2.0% (w/w Cs),
341 Cs-arabinose MR-treated films displayed the lowest WS values of 9.98%, followed by Cs-
342 xylose, Cs-glucose and Cs-fructose MR-treated films ($p < 0.05$), with respective WS of 10.81%,
343 12.05% and 12.19% (**Fig. 1B**). Average WS values of 52% were initially recorded for non-
344 heated films. This increase in MR-treated films insolubility in water, depending on the type and
345 dose of added saccharide, could be the result of higher covalent crosslinking extent, particularly
346 in the presence of aldopentoses. More precisely, this higher WS of the resulting films due to
347 higher glycation rate (the extent of crosslinking between amino groups of Cs and the carbonyl
348 groups of the tested saccharide) could be ascribed to the formation of melanoidins
349 (heterogeneous brown polymers of high molecular weight), non-soluble MR compounds
350 (Stevenson *et al.*, 2020).

351 Based on the latter outcomes, MR, mainly *via* crosslinking with aldopentoses, could be
352 considered as effective alternative allowing the amelioration of Cs-based films water resistance
353 in terms of their insolubility in water.

354 **3.1.3. Water contact angle determination**

355 One of the common ways to measure the wettability (resistance to water absorption) of
356 films surface is the determination of WCA, indicating, the hydrophilicity/hydrophobicity
357 degree of films (Etxabide, Uranga, Guerrero and de la Caba, 2015). Obtained data are reported
358 in **Table 1** and **Supplementary Data Fig. S1**.

359 **Table 1:** Water contact angle (WCA), water vapor (WVP) permeability, tensile strength (TS)
 360 and elongation at break (EAB) of Cs-based MR-treated films, compared to the non-modified
 361 control film.

Film formulations	MR heating time (h)	WCA (°)	WVP	TS (MPa)	EAB (%)	
			10^{-10} (g m ⁻¹ s ⁻¹ Pa ⁻¹)			
Cs	0	92.4±0.6 ^{aA}	3.6±0.1 ^{aB}	18.9±0.5 ^{aA}	29.6±0.4 ^{aA}	
	24	91.5±0 ^{aH}	3.4±0.3 ^{aA}	18.7±0.9 ^{aF}	29.8±0.8 ^{aF}	
Cs-G	0.5	0	92.8±0.2 ^{aA}	3.8±0.9 ^{aAB}	18.6±0.4 ^{aA}	29.9±0.7 ^{aA}
		24	100.8±1 ^{bG}	3±0.4 ^{aA}	19.7±0.3 ^{aE}	30±1 ^{Ac}
	1.0	0	91.4±1 ^{aA}	4.2±0.1 ^{aA}	18.3±0.9 ^{aA}	29.7±0.8 ^{aA}
		24	104.6±0.5 ^{cF}	1.9±0.1 ^{bBC}	21.5±0.2 ^{bD}	31.1±0 ^{aD}
	2.0	0	93.7±1.3 ^{aA}	4.3±0.7 ^{aAB}	19.9±1.5 ^{aA}	29.3±0.6 ^{aA}
		24	109.4±0.4 ^{dF}	1.1±0.2 ^{cC}	23.3±0.3 ^{cB}	33.6±0.5 ^{bBC}
Cs-F	0.5	0	92.4±0.6 ^{aA}	3.9±0.3 ^{aAB}	18.4±0.6 ^{aA}	29.6±0.3 ^{aA}
		24	91.2±1.2 ^{aH}	2.9±0.3 ^{aA}	19.3±0.2 ^{cE}	29.5±0.9 ^{aF}
	1.0	0	92.2±0.2 ^{aA}	4.8±0.2 ^{aA}	18.5±1.1 ^{aA}	29.9±1.4 ^{aA}
		24	101.2±0.5 ^{bG}	2.1±0.2 ^{bB}	21.1±0.5 ^{bD}	29.7±1.2 ^{aF}
	2.0	0	91.2±0.9 ^{aA}	4.9±0.5 ^{aA}	18.9±0.9 ^{aA}	29.8±0.6 ^{aA}
		24	105±1.5 ^{cF}	1.4±0.3 ^{cC}	22.8±0.1 ^{cC}	32.9±0.3 ^{bC}
Cs-X	0.5	0	91.2±0.9 ^{aA}	4.1±0.8 ^{aAB}	19.9±1.5 ^{aA}	29.6±0.6 ^{aA}
		24	108.9±0.1 ^{bE}	2.3±0.1 ^{aB}	19.4±0 ^{aE}	29.5±0.2 ^{aF}
	1.0	0	92.8±0.2 ^{aA}	4.5±0.2 ^{aA}	18.3±1.2 ^{aA}	29.3±1.1 ^{aA}
		24	111.6±0.4 ^{cD}	1.6±0.4 ^{bBC}	22.4±0.1 ^{bC}	32.7±0.6 ^{bC}
	2.0	0	93.9±0.9 ^{aA}	4.2±0.4 ^{aAB}	18.9±0.2 ^{aA}	29.4±0.4 ^{aA}
		24	113.8±0.4 ^{dC}	1.4±0.3 ^{bC}	24.9±0.4 ^{cB}	34.8±0.3 ^{cB}
Cs-A	0.5	0	92.6±0.8 ^{aA}	4.6±0.2 ^{aA}	18.7±0.8 ^{aA}	29.1±0.7 ^{aA}
		24	109.2±0.4 ^{bE}	1.8±0.4 ^{aBC}	21.2±0.6 ^{aD}	32.1±1.1 ^{aC}
	1.0	0	91.4±0.6 ^{aA}	4.4±0.6 ^{aAB}	19.2±0.7 ^{aA}	29.8±0.6 ^{aA}
		24	115.2±1 ^{cB}	1.4±0.2 ^{aC}	23.8±0.2 ^{bB}	34.9±0.9 ^{bB}
	2.0	0	93.2±0.7 ^{aA}	4.6±0.1 ^{aA}	19±0.2 ^{aA}	30±0.4 ^{aA}
		24	118.4±1.5 ^{dA}	1.2±0.2 ^{aC}	26.7±0.8 ^{cA}	37.1±0.8 ^{cA}

362 Different letters (a-e) in the same films group are significantly different as determined by ANOVA test (p<0.05).

363 Different letters (A-H) indicated significant differences between different films groups at the same heating time (p<0.05).

364 Cs: Chitosan, A: Arabinose, F: Fructose, G: Glucose, X: Xylose.

365 No significant difference was shown regarding the WCA of non-heated films and for MR-
366 treated Cs-fructose films at a mass ratio of 0.5% (w/w Cs), with an average WCA value of 92
367 ° ($p>0.05$). Subsequently, overall Cs-based films could be considered as hydrophobic films.
368 Apart from that, as a result of Cs-saccharide MR crosslinking, WCA increased in a saccharide
369 dose dependent manner ($p<0.05$), which could be attributed to the decrease in the hydrophilic
370 character of heated films, as a result of polar groups decrease. For example, in the case of Cs-
371 arabinose MR treated films, WCA values of 109 °, 115 ° and 118 ° were noted at mass ratios
372 of 0.5, 1.0 and 2.0% (w/w Cs), respectively. As well, WCA increased from 100 ° to 109 ° and
373 from 108 ° to 113 °, for Cs-xylose and Cs-glucose mass ratios of 0.5% and 2.0% (w/w Cs),
374 respectively.

375 Taking into consideration these outcomes, the increase in films surface hydrophobicity,
376 reflected by higher WCA values, was the most pronounced in the case of MR crosslinking with
377 aldopentoses (arabinose and xylose), whereas, ketosis (fructose) was the less reactive, showing
378 a relative crosslinking extent, after films heating at 90 °C for 24 h.

379 **3.1.4. Water vapor permeability assessment**

380 The effect of the MR crosslinking on the water barrier properties of Cs-based films was
381 studied, considering films WVP ($10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$) measured at 100% RH and results are
382 summarized in **Table 1**. No significant differences in the WVP values were noted for the non-
383 heated and heated free-saccharide Cs-based film, as well as non-heated saccharides-added films
384 ($p>0.05$). However, WVP of heated films significantly decreased with the addition of
385 saccharides to the film forming solutions, compared to the blank Cs-based film ($3.4 \cdot 10^{-10} \text{ g m}^{-1}$
386 $\text{s}^{-1} \text{ Pa}^{-1}$). This decrease was saccharide type and dose dependent, where films crosslinked with
387 aldopentoses (less carbons in their macromolecular chain, with a carbonyl group at the end of
388 the carbon chain) displayed the lowest WVP ($p<0.05$). Values of $1.4 \cdot 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ and 1.6
389 $\cdot 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ were reached with 1.0% (w/w Cs) of arabinose and xylose, respectively. The

390 lowest reactivity, expressed in terms of the highest WVP value of $2.1 \cdot 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, was
391 observed with the Cs-fructose crosslinked films (ketosis), at the same concentration.
392 Additionally, WVP decreased from 1.8 to $1.2 \cdot 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, when the concentration of
393 arabinose increased from 0.5 to 2.0% (w/w Cs). The same trend was noted with the xylose,
394 fructose and glucose ($p < 0.05$).

395 The decrease in the WVP could be ascribed to the reduction of the films network free
396 volume (films pore size), subsequently to the MR crosslinking. This allowed a sufficient
397 strengthening of the polymeric network, and thereby, the enhancement of Cs-MR-treated films
398 mechanical properties. Therefore, a significant effect on the diffusion of water, among very
399 small molecules (Hazaveh *et al.*, 2015). Serrano-Leon *et al.* (2018) stated that the film barrier
400 properties are affected by the filmogenic matrix moisture content. Indeed, a decrease in water
401 concentration results in a decrease in molecular chains' mobility, ultimately changing
402 permeability behavior of the MR-treated films.

403 **3.2. Effect of the MR on films color and light barrier properties**

404 **3.2.1. Study of films color parameters**

405 Optical features, including color, besides physicochemical and thermal behavior, are a
406 key character to substantiate the effectiveness of packaging intended to food applications (Jiang
407 *et al.*, 2016). Visually, no significant modification was noted regarding non-heated blank and
408 Cs-saccharides films color (**Supplementary Data Fig. S2**). Whereas, the color of MR
409 crosslinked films shifted from colorless to dark yellow to variable extent, depending on the type
410 and dose of incorporated saccharide. Therefore, changes in color of blank and MR-treated Cs-
411 based films were determined and shown in **Table 2**. L^* (whiteness/darkness), a^*
412 (redness/greenness) and b^* (yellowness/blueness) values were used for the quantification of
413 films color. Higher L^* , negative a^* and b^* values imply lightness, green and blue color,
414 respectively, while positive a^* and b^* values indicate red and yellow color, respectively.

415 In the present study, L* values decreased with the increase of saccharide content in the
416 Cs-film forming solution, reaching a minimum of 85% in the presence of 2.0% (w/w Cs) of
417 arabinose, after a 24 h heating time at 90 °C (p<0.05). Values of 88% and 87% were revealed
418 with arabinose mass ratios of 0.5 and 1.0% (w/w Cs). While, negligible variations of lightness
419 were detected for non-heated films and even for heated blank film (p>0.05), proving that color
420 was not mainly affected by heating. The higher L* were displayed in the presence of fructose
421 (ketosis), showing relative crosslinking rate, since the developpement of dark products is
422 correlated with the extent of Cs-saccharide interaction through the MR. L* values of 90%, 89%
423 and 88% were recorded for 0.5, 1.0 and 2.0% (w/w Cs) mass ratios of fructose. Lipid oxidation
424 and food quality are mainly influenced by packaging material opacity, which is a key feature to
425 substantiate the effectiveness of packaging intended to food applications as a protective agent
426 of food materials from degradative effects of light. Indeed, light, specifically UV light, is mainly
427 involved in the oxidation process, by chemical reactions catalyzation, which accelerates the
428 deterioration of food, affecting ultimately its acceptability by the consumer (Su *et al.*, 2012).

429 In addition, MR led to a significant intensification of Cs-based films dark yellowish color
430 as illustrated by b* values measurements (**Table 2**). Indeed, b* increased significantly from 3.6
431 for blank Cs-based film to 10.9, 21.5, 25.7 and 31.6 for 2.0% (w/w Cs) content of fructose,
432 glucose, xylose and arabinose, respectively. Even at the least mass ratio of 0.5% (w/w), a
433 significant and rapid improvement in films yellowish color, by 2, 2.4, 2.6 and 3-folds, was
434 observed for fructose, glucose, xylose and arabinose, respectively. In line with L* results,
435 ketosis (fructose) was the least reactive, while aldopentoses (particularly arabinose) showed the
436 highest MR crosslinking extent. A different tendency was noted regarding the a* coordinates,
437 where values decreased toward the green region with MR crosslinking, depending on the
438 saccharide type and dose (**Table 2**). Equally, no significant changes were detected for the non-
439 heated films and the blank Cs-based film (p>0.05).

Table 2: Color parameters, browning index (BI) and transparency of Cs-based MR-treated films, compared to the non-modified control film.

Film formulations	MR heating time (h)	Color parameters				BI	Transparency	
		L*	a*	b*	ΔE			
Cs	0	91.2±0.4 ^{aA}	2.4±0.1 ^{aA}	3.6±0.3 ^{aA}	-	-	0.9±0.1 ^{aA}	
	24	91.2±0.4 ^{aA}	2.4±0.1 ^{aA}	3.6±0.3 ^{aA}	-	-	1±0.1 ^{aA}	
Cs-G	0.5	0	91.3±0.7 ^{aA}	2.4±0.2 ^{aA}	3.6±0.6 ^{aA}	-	-	0.9±0 ^{aA}
		24	89.1±0.1 ^{bC}	-0.9±0 ^{bB}	8.8±0.4 ^{bC}	5.9±1 ^{cBC}	9.3±0.5 ^{aC}	2.6±0.1 ^{bC}
	1.0	0	91.8±0.3 ^{aA}	2.3±0.1 ^{aA}	3.4±0.5 ^{aA}	-	-	0.9±0.1 ^{aA}
		24	88.5±0.1 ^{cC}	-1.4±0.1 ^{cC}	11.3±0.7 ^{cC}	9.1±0.6 ^{bC}	12±0.8 ^{bC}	3.8±0.1 ^{cD}
	2.0	0	91.2±0.4 ^{aA}	2.3±0.5 ^{aA}	3.5±0.2 ^{aA}	-	-	1±0.1 ^{aA}
		24	87.1±0.4 ^{dC}	-2.1±0 ^{dC}	21.5±1 ^{dC}	19±0.8 ^{aC}	25.5±1.3 ^{cC}	4±0 ^{dC}
Cs-F	0.5	0	91.3±0.5 ^{aA}	2.4±0.8 ^{aA}	3.6±0.3 ^{aA}	-	-	0.9±0.2 ^{aA}
		24	90.6±0.2 ^{bB}	-0.9±0.1 ^{bB}	7.5±0.4 ^{bB}	5.1±0.4 ^{cC}	7.6±0.4 ^{cD}	2.3±0.2 ^{bC}
	1.0	0	91.6±0.9 ^{aA}	2.5±0.1 ^{aA}	3.4±0.4 ^{aA}	-	-	0.9±0 ^{aA}
		24	89.5±0.2 ^{cB}	-1.2±0.1 ^{cB}	9.2±0.3 ^{cB}	7±0.3 ^{bD}	9.5±0.3 ^{bD}	3.5±0.1 ^{cC}
	2.0	0	90.9±0.6 ^{aA}	2.5±0.4 ^{aA}	3.5±0.7 ^{aA}	-	-	1±0.1 ^{aA}
		24	88.2±0.1 ^{dB}	-1.4±0.1 ^{dB}	10.9±0.6 ^{dB}	8.8±0.5 ^{aD}	11.5±0.7 ^{aD}	4±0.1 ^{dC}
Cs-X	0.5	0	91.3±0.8 ^{aA}	2.4±0.2 ^{aA}	3.6±0.3 ^{aA}	-	-	1±0.1 ^{aA}
		24	88.6±0.1 ^{bD}	-0.9±0 ^{bB}	9.4±0.3 ^{bC}	7.2±0.3 ^{cB}	10.1±0.3 ^{cB}	3±0 ^{bB}
	1.0	0	92±0.7 ^{aA}	2.4±0.1 ^{aA}	3.6±0.2 ^{aA}	-	-	1±0.1 ^{aA}
		24	87.2±0.1 ^{cD}	-2.7±0.1 ^{cD}	13±0.4 ^{cD}	11.5±0.4 ^{bB}	13.3±0.8 ^{bB}	4.7±0.1 ^{cB}
	2.0	0	92.1±0.9 ^{aA}	2.5±0.3 ^{aA}	3.6±0.1 ^{aA}	-	-	1±0 ^{aA}
		24	86.3±0.6 ^{dD}	-3.4±0.2 ^{dD}	25.7±0.4 ^{dD}	23.4±0.2 ^{aB}	30.9±0.6 ^{aB}	5.5±0 ^{dB}
Cs-A	0.5	0	91.3±0.9 ^{aA}	2.4±0.4 ^{aA}	3.6±0.1 ^{aA}	-	-	1±0.1 ^{aA}
		24	88.5±0.5 ^{bD}	-1.4±0.1 ^{bC}	11.1±0.3 ^{bD}	8.9±0.1 ^{cA}	11.7±0.3 ^{cA}	4±0.1 ^{bA}
	1.0	0	91.9±0.7 ^{aA}	2.4±0.3 ^{aA}	3.6±0.6 ^{aA}	-	-	1±0 ^{aA}
		24	87.2±0.3 ^{cE}	-2.8±0.2 ^{cD}	16.2±0.5 ^{cE}	14.3±0.6 ^{bA}	17.5±0.6 ^{bA}	5.2±0.1 ^{cA}
	2.0	0	92.2±0.5 ^{aA}	2.4±0.4 ^{aA}	3.7±0.4 ^{aA}	-	-	1.1±0 ^{aA}
		24	85.9±0.4 ^{dE}	-3.8±0.1 ^{dE}	31.6±0.5 ^{dE}	29.2±0.4 ^{aA}	40.6±0.5 ^{aA}	6.2±0.1 ^{dA}

~~042~~ Different letters (a-d) in the same films group are significantly different as determined by ANOVA test (p<0.05).

~~043~~ Different letters (A-E) indicated significant differences between different films groups at the same heating time (p<0.05).

~~044~~ C: Chitosan, A: Arabinose, F: Fructose, G: Glucose, X: Xylose.

445 To better study the above-mentioned differences between blank and MR-treated films,
446 ΔE was determined (**Table 2**). Similarly, ΔE shapely increased with the increase of saccharide
447 to Cs mass ratio, especially in the presence of aldopentoses, reaching values of 8.8, 19, 23 and
448 29 for fructose, glucose, xylose and arabinose, respectively ($p < 0.05$).

449 Additionally, the BI, reflecting the extent of MR colored products developpement, was
450 assessed. Results in **Table 2** show the BI variation of Cs-saccharide MR-conjugated films as a
451 function of saccharide incorporated mass. Indeed, BI increased uniformly with the increase of
452 saccharide content ($p < 0.05$). For example, values reached more than 11, 17 and 40 for 0.5, 1.0
453 and 2.0% (w/w Cs) of arabinose, respectively. The highest BI values were noted in the presence
454 of aldopentoses (xylose and especially arabinose), followed by glucose (aldohexoses). The
455 lowest MR progress, in terms of the lowest BI values, was attained with fructose (ketosis),
456 which is in line with ΔE and b values. Such findings proved that Cs-MR-conjugated films are
457 dark with yellowish color, attributed to the developpement of MR colored products, which
458 could result in an enhancement of their light barrier ability in the visible range.

459 Furthermore, it was found that the same trend of variation in terms of total color
460 difference and water solubility was followed as a function of MR crosslinking extent
461 (saccharide type and dose). In fact, the MR could be summarized as follows: at the early stage
462 of the MR, conjugates (Schiff base) between the carbonyl group of saccharides and the amine
463 group of Cs are formed, and subsequently cyclizes to develop the Amadori compounds.
464 Thereafter, melanoidins, colored and insoluble polymeric compounds, are formed, allowing the
465 noticeable drop of the Cs-MR-crosslinked films WS and the increase of their BI (Stevenson *et*
466 *al.*, 2020).

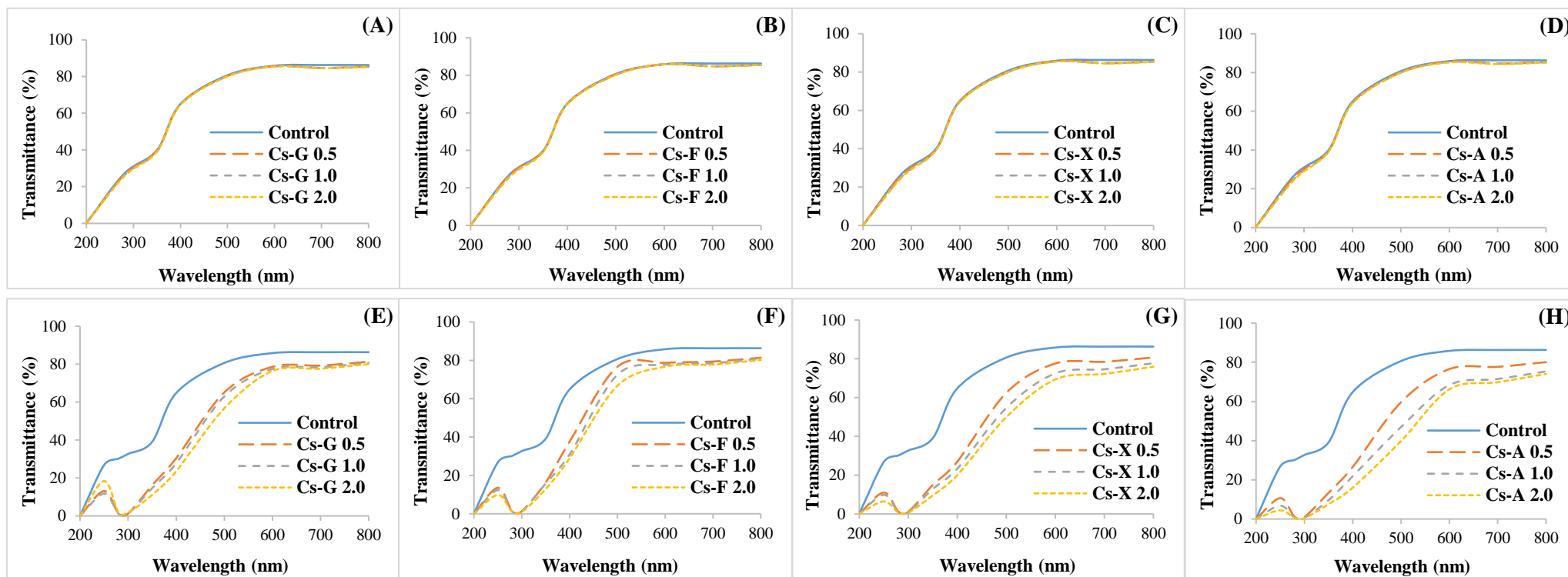
467 **3.2.2. Light barrier properties assessment**

468 Light, specifically UV light, is mainly implicated in the oxidation process, through
469 chemical reactions catalyzation, hastening food deterioration, and thereby, its acceptability by

470 the consumer (Mahmoudi, Ostadhossein and Simchi, 2016). Therefore, UV and visible light
471 transmission by Cs-MR-conjugated films was assessed in a wavelength range of 200–800 nm
472 and compared with the blank Cs-based film behavior. Results, plotted in **Fig. 2**, reveal similar
473 trends of variation as function of the MR extent with b^* , ΔE and BI, ascribed to the progress of
474 yellow-brown MR end products (melanoidins).

475 Blank Cs film exhibited a poor barrier property to the light in the UV range as observed
476 through high transmission values at 280 and 350 nm, of 26.97% and 39.81%, respectively. Non-
477 heated films, added with saccharides, showed no significant variations in the light barrier
478 properties of the in terms of the transmittance values (**Fig. 2A-D**). In the range of 250–400 nm,
479 Cs-MR-conjugated films spectra revealed particular absorption, in terms of light transmission
480 values that decreased remarkably ($p < 0.05$) (**Fig. 2E-H**). This is assumed to be ascribed to the
481 light scattering or the interfering of light track, subsequently to Cs-saccharide crosslinking,
482 hindering thereby light transmittance (Kaya *et al.*, 2018). In fact, oxidation process mainly
483 involves light, specifically UV light, which generates chemical reactions catalyzation, and
484 accelerates the deterioration of food, affecting ultimately the consumer acceptance (Su *et al.*,
485 2012).

486 The noteworthy light transmittance reduction at 280 nm, for Cs-MR-treated films
487 reaching an average value of 1%, vs. 30% for blank Cs-based film, could be attributed to the
488 presence of carbonyl compounds and heterocyclic derivatives (Etxabide *et al.*, 2015). This
489 remarkable UV light barrier increased concomitantly with the increase of saccharide to Cs mass
490 ratio, indicating that Cs-MR-conjugated films effectively prevented UV light. The better barrier
491 behavior, reflected by the lowest light transmittance values, was, as expected, observed with Cs
492 crosslinked with aldopentoses (arabinose and xylose), indicating higher MR products
493 developpement, compared to crosslinking with aldohexoses (glucose) and ketosis (fructose).
494 The lower the transmittance value, the less transparent the films are (Souza *et al.*, 2017).



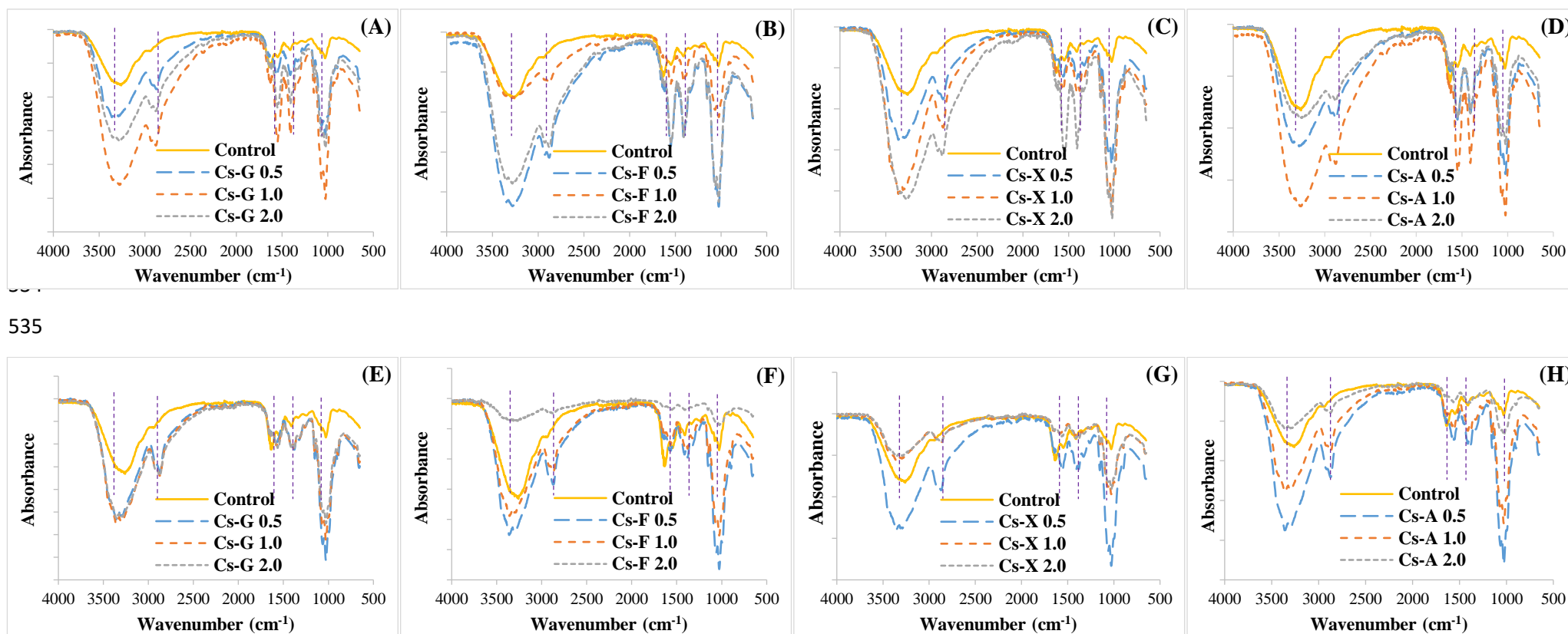
509 **Figure 2:** Transmittance of Cs-based films conjugated with different saccharides, at different mass ratios (0.5, 1.0 and 2.0 %; w/w Cs), through
 510 MR at 90 °C as a function of time (0 and 24 h). Non heated (A-D) and Heated (E-H) Cs-based films. Cs: Chitosan, A: Arabinose, F: Fructose, G:
 511 Glucose, X: Xylose.

512 Compared to the blank Cs-based film and Cs-saccharides non-heated films (average
513 opacity of 0.9), the opacity values for the Cs-MR-crosslinked films were significantly higher
514 ($p < 0.05$), especially with aldopentoses (arabinose and xylose), indicating a lower transparency
515 of the films (**Table 2**).

516 Cs-arabinose conjugated film at a mass ratio of 2.0% (w/w Cs) showed an enhancement
517 in opacity by more than 6-folds, as compared to blank Cs-based film ($p < 0.05$). This finding
518 heightens the application of Cs-MR-conjugated films in food preservation (Stevenson *et al.*,
519 2020; Mahmoudi, *et al.*, 2016).

520 **3.3. Infrared spectroscopic analysis**

521 To further confirm the crosslinking in Cs-based films induced by the MR, besides
522 chemical changes of Cs structure due to saccharide addition, FT-IR spectra of non-heated and
523 heated Cs-saccharide films were studied and resulting patterns are shown in **Fig. 3**. Typical
524 characteristic Cs absorption bands were revealed at around 1589 cm^{-1} attributed to N-H bending
525 in the NH-COCH_3 group (amide II band), 1649 cm^{-1} characteristic of the amide I band (C=O in
526 the NHCOCH_3 group), 1418 cm^{-1} indicating the C-H bending vibrations of CH_2 , 2905 cm^{-1}
527 ascribed as CH_2 and CH_3 groups, 894 cm^{-1} assigned to the absorption peaks of β -(1,4) glycosidic
528 bands and at 3423 cm^{-1} attributed to the stretching vibrations of the OH and N-H groups (Hamdi
529 *et al.*, 2019b). additionally, for heated blank Cs-based film and non-heated Cs-saccharides films
530 (**Fig. 3A-D**), no substantial modification of FT-IR spectra compared to the blank non-heated
531 Cs-based film were detected. In addition, for all studied film samples, a glycerol characteristic
532 band was detected at wavenumber 1046 cm^{-1} , which corresponded to the OH group vibrations
533 (Vlacha *et al.*, 2016).



535

537 **Figure 3:** ATR -FTIR spectra of Cs-based films conjugated with different saccharides, at different mass ratios (0.5, 1.0 and 2.0 %; w/w Cs),
 538 through MR at 90 °C as a function of time (0 and 24 h). Non heated (A-D) and Heated (E-H) Cs-based films. Cs: Chitosan, A: Arabinose, F: Fructose,
 539 G: Glucose, X: Xylose.

540 The Cs and saccharides' intermolecular interactions were illustrated by the shifts in band
541 positions (Stevenson *et al.*, 2020; Xu *et al.*, 2019; Ahmed and Ikram, 2016). Indeed, after
542 reaction, independently of their type and concentration, saccharides addition to Cs caused
543 significant difference in the resulting spectra (**Fig. 3E-H**). The absorption peak at wavenumber
544 1649 cm^{-1} (Amide I) decreased and shifted to 1607 cm^{-1} suggesting the formation of Schiff
545 base ($-\text{C} = \text{N}$) between the saccharides reducing termination and the Cs amino groups (Zhong
546 *et al.*, 2019). In brief, the MR conjugation between an aldose sugar and an amino group of Cs
547 stimulated the developpement of N-substituted glycosylamines that arranged to Amadori
548 rearrangement products, the 1 amino-1-desoxy-2-ketoses. Thus, the formation of Schiff base of
549 furfurals involved in aldols, aldimines, ketimes and melanoidins formation (Duconseille,
550 Astruc, Quintana, Meersman and Sante-Lhoutellier, 2015).

551 Moreover, shifts from 1589 cm^{-1} (Amide II) to 1544 cm^{-1} , after 24 h MR time, were
552 ascribed to the Cs conformation transformations, during the crosslinking reaction between Cs
553 and saccharides. After MR crosslinking, more randomly disposition occurred, increasing,
554 thereby, the molecular disorder (Badano *et al.*, 2019). In fact, the reaction of the carbonyl group
555 of each saccharide with the amino group of Cs, resulted in the elaboration of C-O-C ether
556 linkage, due to -OH condensation. Therefore, overall change in Cs-saccharides glycated system
557 took place and a characteristic adsorption in the wavenumber range of $1098\text{-}1021\text{ cm}^{-1}$ was
558 detected (Du, Huang, Wang and Xiao, 2018).

559 **3.4. Thermal behavior of Cs-based films as affected by the MR crosslinking**

560 The thermal stability of Cs-based films crosslinked or not with the MR was assessed by
561 TGA in a temperature range of 25 to $800\text{ }^{\circ}\text{C}$ (**Supplementary Data Fig. S3**). The thermal
562 decomposition data in terms of corresponding weight loss (Δw), temperature of maximum
563 degradation (T_{max}) and final residual mass (R) are reported in **Table 3**.

564

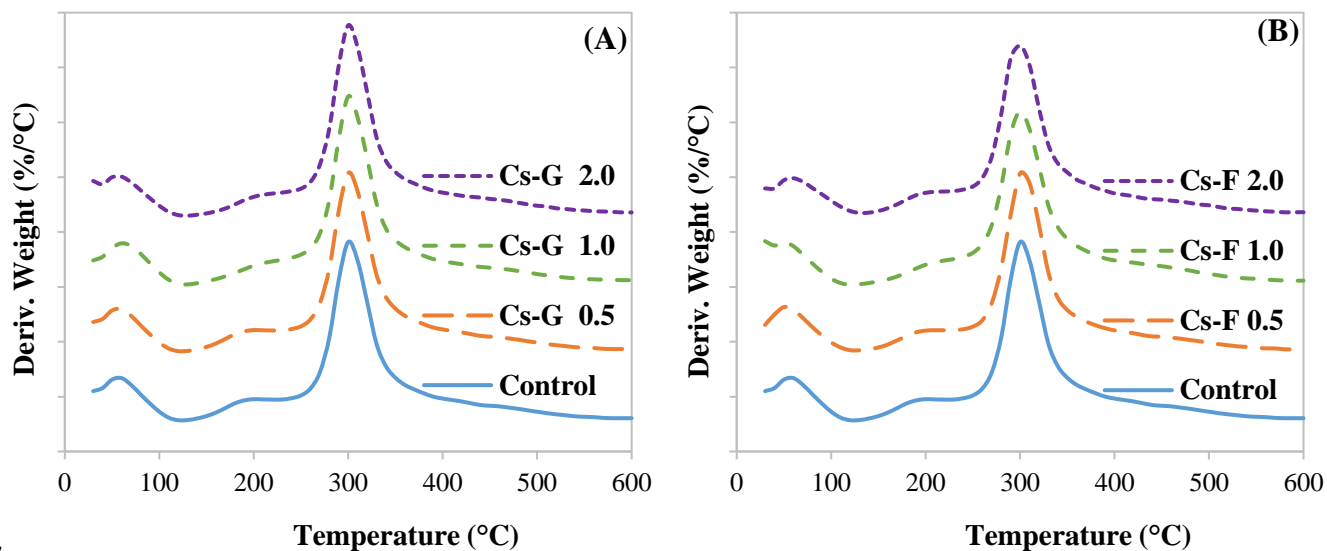
565 **Table 3:** Weight loss (Δw), temperature of maximum degradation (T_{max}), residual mass (R)
 566 and glass transition temperature (T_g) of Cs-based films crosslinked with different saccharides
 567 of different molar ratios, *via* the MR at 90 °C.

Film formulations	MR heating time (h)	Δw (%)	T_{max} (°C)	R (%)	T_g (°C)	
Cs	0	67.2 ^{aA}	294.3 ^{aA}	25.9 ^{aA}	162.6 ^{aA}	
	24	66.6 ^{bA}	292.8 ^{bA}	26.8 ^{aA}	169.9 ^{bA}	
Cs-G	0.5	0	67.8 ^{aB}	296.7 ^{aC}	26 ^{aA}	162.4 ^{aA}
		24	66.8 ^{bA}	299.6 ^{cC}	30.2 ^{bB}	182.6 ^{cC}
	1.0	0	67.1 ^{aB}	297.9 ^{bD}	26.1 ^{aA}	163.2 ^{bA}
		24	66.3 ^{bA}	301.2 ^{dE}	30.6 ^{bB}	183.1 ^{dC}
2.0	0	66.4 ^{bC}	299.2 ^{cF}	25.8 ^{aA}	162.9 ^{aA}	
	24	65.9 ^{cB}	302 ^{eF}	31.4 ^{cB}	187.6 ^{eD}	
Cs-F	0.5	0	69 ^{aA}	295.6 ^{aB}	25.9 ^{aA}	162.8 ^{aA}
		24	66.3 ^{cA}	298 ^{cB}	30.1 ^{bB}	179.1 ^{bB}
	1.0	0	67.1 ^{bB}	297.4 ^{bD}	26.1 ^{aA}	162.5 ^{aA}
		24	65.8 ^{dB}	298.9 ^{cdB}	30.6 ^{bB}	181.1 ^{cB}
	2.0	0	66.2 ^{cC}	298.8 ^{cdE}	26.1 ^{aA}	162.1 ^{aA}
		24	65.6 ^{dB}	300.4 ^{dD}	31.2 ^{bB}	184.6 ^{dB}
Cs-X	0.5	0	67.4 ^{aB}	298 ^{aE}	26.1 ^{aA}	162.3 ^{aA}
		24	66.3 ^{bA}	299.6 ^{bcC}	30.4 ^{bB}	183.4 ^{bD}
	1.0	0	67.4 ^{aB}	299.7 ^{bcF}	26 ^{aA}	162.9 ^{aA}
		24	66.3 ^{bA}	301.2 ^{dE}	31.5 ^{cC}	186.7 ^{cE}
	2.0	0	66.5 ^{bC}	300.4 ^{cG}	26.1 ^{aA}	162.7 ^{aA}
		24	65.1 ^{cB}	302.8 ^{eF}	32.2 ^{dC}	188.4 ^{dC}
Cs-A	0.5	0	67.6 ^{aB}	299.3 ^{aF}	25.9 ^{aA}	162.6 ^{aA}
		24	66.5 ^{bA}	300.4 ^{bD}	30.6 ^{bB}	183.9 ^{cD}
	1.0	0	66.4 ^{bC}	301.2 ^{cH}	26.1 ^{aA}	163.2 ^{bB}
		24	65.7 ^{cB}	302.1 ^{dE}	31.9 ^{cC}	185.8 ^{dD}
	2.0	0	64.9 ^{dD}	301.9 ^{cdH}	26.2 ^{aA}	163.1 ^{bB}
		24	63.8 ^{eC}	303.6 ^{eG}	32.8 ^{dC}	193.1 ^{eE}

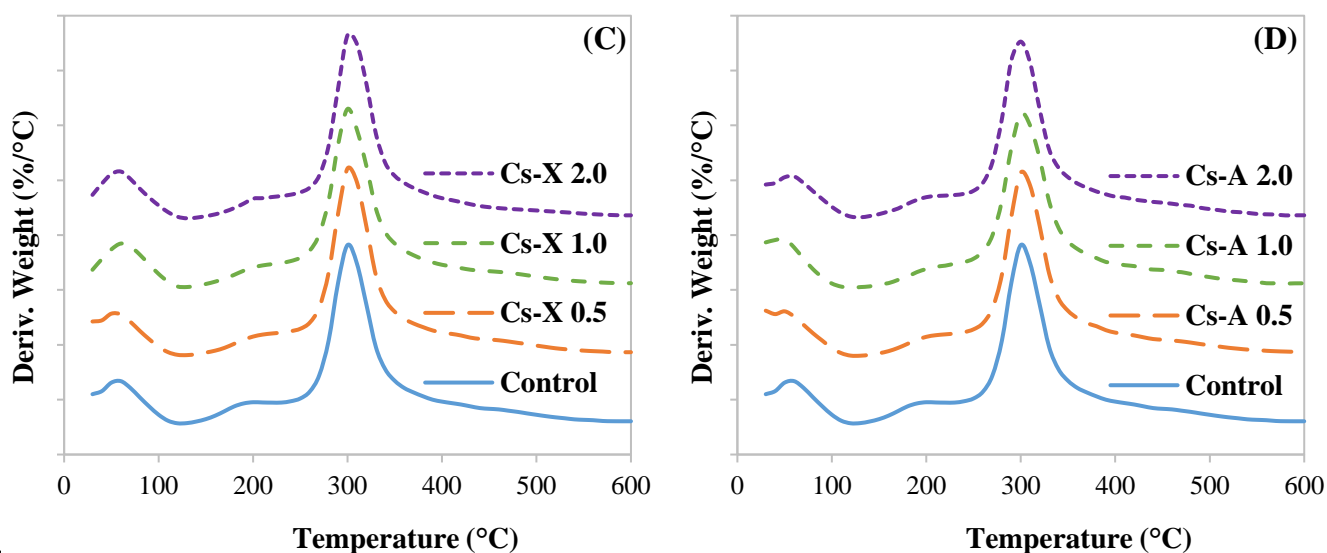
568 Different letters (a-e) in the same films group are significantly different as determined by ANOVA test ($p < 0.05$).
 569 Different letters (A-H) indicated significant differences between different films groups at the same heating time ($p < 0.05$). **Cs:**
 570 Chitosan, **A:** Arabinose, **F:** Fructose, **G:** Glucose, **X:** Xylose; **T_{max}:** Temperature of the maximum degradation of the major
 571 transformation region; **R:** Residue.

572

57



57



575 **Figure 4:** DTG profiles of Cs-based films conjugated with different saccharides, at different
576 mass ratios (0.5, 1.0 and 2.0 %; w/w Cs), through MR at 90 °C for 24 h. Cs: Chitosan, A:
577 Arabinose, F: Fructose, G: Glucose, X: Xylose.
578

579 Based on data from the obtained TGA thermograms, the thermal decomposition profiles
580 of the overall films exhibited a similar weight loss process in the temperature range of 25 to
581 800 °C, characterized by two distinguishable major phases (**Fig. 4**), characteristic of chitosan-
582 based biomaterials thermal decomposition. The first stage of transformation is related to the
583 loss of free and bound water (below 100 °C), which tended to decrease for Cs-MR-crosslinked
584 films, in line with the WC findings (**Fig. 1A**), proving that MR-treated films (90 °C, 24 h)

585 exhibited lower WC than the non-heated films. The second stage of weight loss, corresponding
586 to the major Δw around 63 to 67%, displayed the degradation or the decomposition of Cs chains
587 at approximately 292-303 °C.

588 Results in **Table 3** display that the incorporation of saccharides in the Cs film-forming
589 matrix without heat treatment did not affect significantly the Δw of films, at around 67%.
590 Nevertheless, the lowest Δw value was around 63% for Cs-arabinose crosslinked film, at a mass
591 ratio of 2.0% (w/w Cs).

592 Regarding T_{max} values, no significant changes were noted for the non-heated films
593 ($p>0.05$). However, when comparing to the blank heated Cs-based film (292 °C), saccharides
594 incorporation increased T_{max} values of Cs-MR-conjugated films in a saccharide dose
595 dependent manner ($p<0.05$), to reach by 300.4 °C, 302 °C, 302.8 °C and 303.6 °C for Cs-
596 fructose, Cs-glucose, Cs-xylose and Cs-arabinose crosslinked films, at a saccharide to Cs mass
597 ratio of 2.0% (w/w Cs), respectively (**Table 3**).

598 Similar trends were observed for the R values, which raised concomitantly with the
599 saccharide concentration, attaining a maximum R value of 32% with 2.0% (w/w Cs) of
600 aldopentoses (arabinose). This thermal resistance behavior could be correlated to the MR
601 crosslinking extent, through creation of new bonds between Cs macromolecular chains,
602 inducing their thermal stability. Additionally, MR products could interact with the Cs polymeric
603 matrix, allowing the stabilization of film network, thus the increase in the observed thermal
604 stability for Cs-MR-treated films (Kchaou *et al.*, 2018; Su *et al.*, 2012).

605 The thermal properties of Cs-MR-conjugated films were further assessed by the DSC
606 analysis in order to determine the glass transition temperature (T_g) and obtained results are
607 shown in **Table 3**. In the same line with TGA outcomes, a clear increase in all films T_g values
608 was displayed with increasing the saccharide content, which could be ascribed either to WC
609 decrease, or to crosslinking phenomenon. Indeed, the T_g increased from 169 °C for blank Cs-

610 based film to 184, 187, 188 and 193 °C for Cs-fructose, Cs-glucose, Cs-xylose and Cs-arabinose
611 crosslinked films, at a saccharide to Cs mass ratio of 2.0% (w/w Cs), respectively (p<0.05).
612 Even at low saccharide content of 0.5% (w/w Cs), improved Tg values of 179, 182, 183.4 and
613 183.9 °C for Cs-fructose, Cs-glucose, Cs-xylose and Cs-arabinose crosslinked films,
614 respectively (p<0.05). Advantageously, DSC thermograms (**Supplementary Data Fig. S4**)
615 reveal a single Tg for all the tested films indicating a good compatibility between tested
616 saccharides and Cs, in these conditions (Hamdi *et al.*, 2019a).

617 Consequently, the Tg as well as the degradation temperature enhancement approved that
618 MR, through its crosslinking effect between the amino group of the glucosamine unit and the
619 carbonyl of saccharide, modified the Cs polymeric structure, elaborating a more thermally
620 stable matrix. This phenomenon is more pronounced in the presence of pentoses (arabinose and
621 xylose), with less carbons in their macromolecular chain, showing higher crosslinking extent,
622 resulting in higher degradation temperatures values, compared to the hexoses (fructose and
623 glucose), with more carbons. Particularly, aldoses (arabinose, xylose and glucose), with a
624 carbonyl group located at one end of the carbon chain, were more reactive than ketoses
625 (fructose), with a carbonyl group situated inside the carbon chain. This enhanced and interesting
626 thermal behavior of Cs-MR-conjugated films could be promising for upgraded mechanical
627 properties.

628 **3.5. Mechanical properties changes of Cs-based MR-treated films**

629 To have information about their flexibility and stretchability, mechanical properties of
630 Cs-saccharides crosslinked through the MR, in terms of TS and EAB were measured and
631 compared to Cs-based film (**Table 1**). Indeed, used or predicted materials for food packaging
632 need to uphold their integrity and effectively resist to plenty types of stress occurring during
633 distribution, treatment and storage (Kaya *et al.*, 2018). Based on the tensile stress *vs.* strain
634 curves, no significant effect of the addition of saccharides without heating, i.e. no MR occurred,

635 on the control films TS and EAB values was observed ($p>0.05$). In addition, heat treatment (90
636 °C for 24 h) did not affect the mechanical parameters of Cs-blank film ($p>0.05$).

637 In counterpart, subsequently to the MR crosslinking, a significant improvement of Cs-
638 saccharides MR crosslinked films mechanical behavior was detected, considering the resultant
639 increase of TS and EAB values, in a saccharide dose-dependent manner ($p<0.05$). In fact, TS
640 values enhanced by 1.17, 1.28 and 1.45-folds, for 0.5, 1.0 and 2.0% (w/w Cs) of arabinose,
641 compared to the blank Cs-based film (18.7 MPa). Equally, EAB increased from 29.8% for blank
642 Cs-based film to 32.1% and 37.1% for 0.5% and 1.0% (w/w Cs) Cs-arabinose crosslinked films,
643 respectively (**Table 1**). The same trend was noted for the other tested saccharides, but in the
644 following order of reactivity: arabinose > xylose > glucose > fructose. The lowest TS value of
645 22.8 MPa and EAB of 32.9% were exhibited by the 2.0% (w/w Cs) Cs-fructose MR crosslinked
646 film.

647 These advantageous mechanical properties of Cs-saccharides MR crosslinked films,
648 particularly through conjugation with aldopentoses, among which arabinose and xylose, is in
649 line with thermal (DSC and TGA) outcomes, proving a positive correlation between TS and Tg
650 of films. Actually, the changes of Tg is not only a current indicator of additives and
651 macromolecules compatibility in the film polymeric network, but it permits likewise to
652 enlighten the polymeric material mechanical resistance parameters (Mujeeb Rahman, Abdul
653 Mujeeb, Muraleedharan and Thomas, 2018). This particular behavior (more reactivity with
654 aldopentoses than ketoses and hexoses) corroborated, as well, with the above described MC
655 results, attributed to MR crosslinking, besides the saccharides plasticizing action (Kamboj *et*
656 *al.*, 2015).

657 **3.6. Effect of the MR crosslinking on the microstructure of Cs-based films**

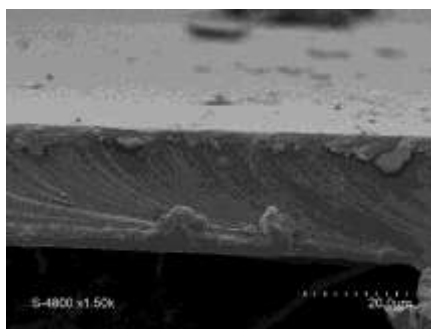
658 The Cs-based films treated with the MR were analyzed for transverse sections after gold
659 coating, to qualitatively visualize their microstructure (homogeneity, layer, voids, etc.) and to

660 identify microstructural modifications, allowing a better understanding of polymers film-
661 forming behavior.

662 Based on the overall above reported results, the Cs-saccharides films at a concentration
663 of 2.0% (w/w Cs) crosslinked through the MR (90 °C for 24 h) were selected for the SEM
664 analysis, and compared with the non-added Cs-based film (**Fig. 5**).

665

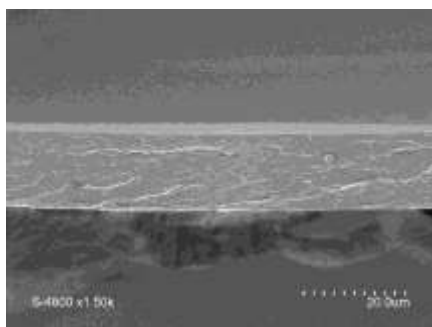
Cs



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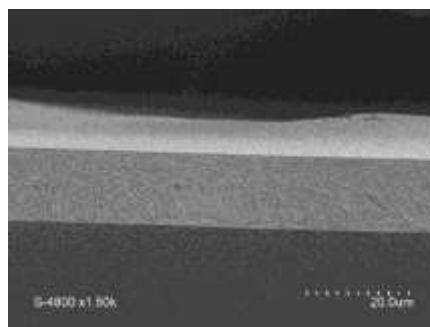
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Cs-G



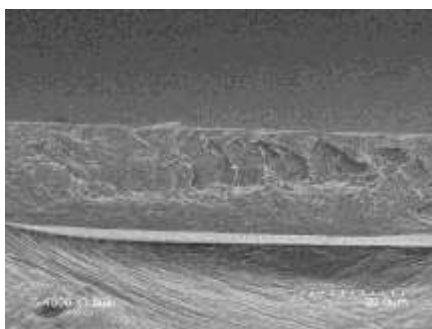
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Cs-F



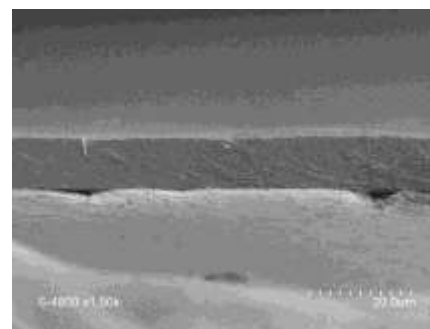
669

Cs-X



670

Cs-A



671 **Figure 5:** SEM cross-section (1.50 kV, 20 μm and 25.0 kV) of Cs-based films conjugated
672 with different saccharides, at different mass ratios (0.5, 1.0 and 2.0 %; w/w Cs), through MR
673 at 90 °C for 24 h. Cs: Chitosan, A: Arabinose, F: Fructose, G: Glucose, X: Xylose.

674 Characteristic micrographs of films cross-sections of films are shown in **Fig. 5**, where, a
675 continuous, homogeneous phase in Cs-based film could be observed, without any evidence of
676 irregularities (air bubbles, pores, cracks or droplets) or phase separation (Hamdi *et al.*, 2019a).

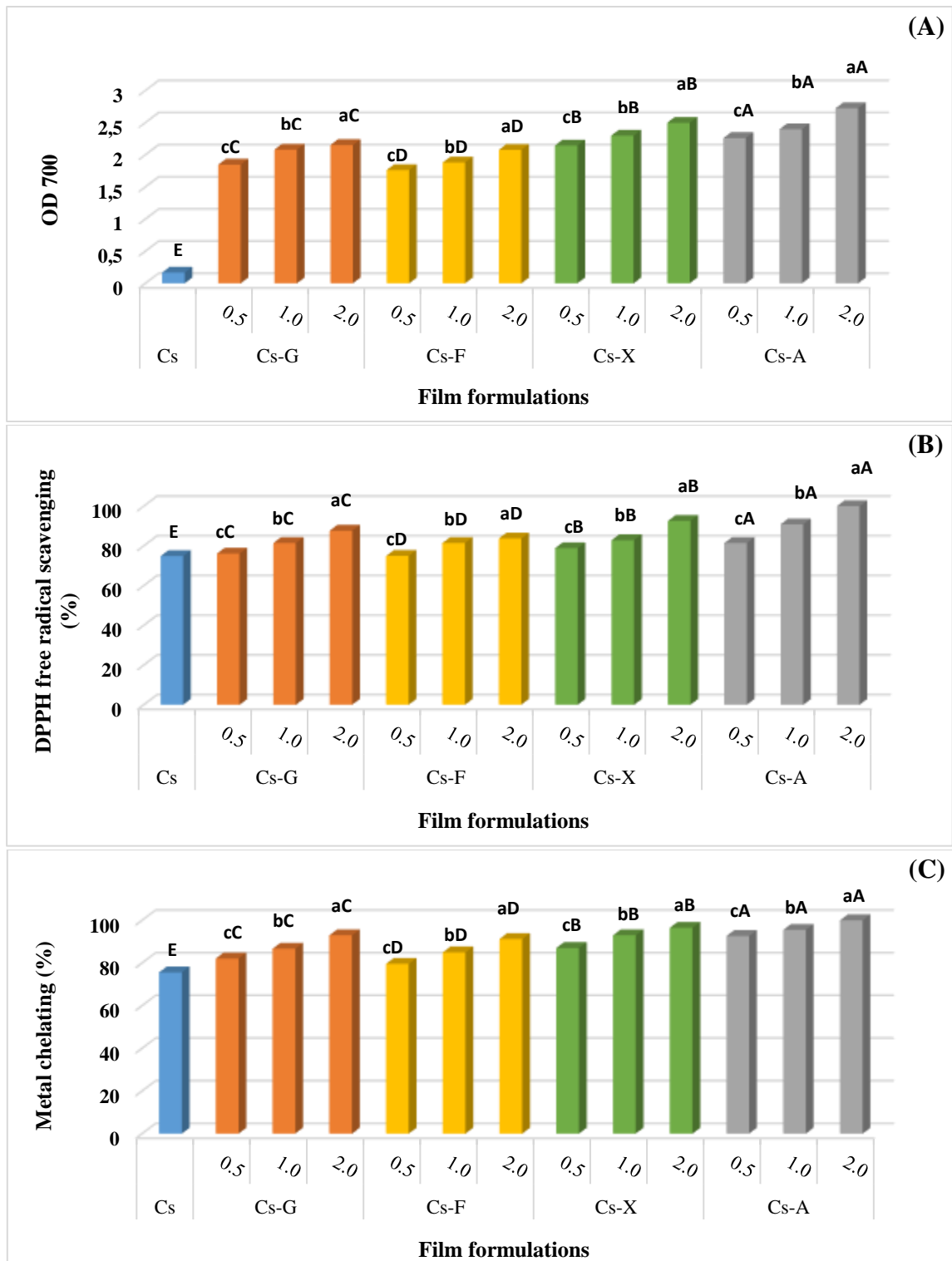
677 The incorporation of saccharides into Cs network combined with heat treatment at 90 °C
678 for 24 h distinguishably changed the cross-section structure of MR-treated films. It was possible
679 to confirm more homogeneity and density were detected with higher crosslinking rates.
680 Otherwise, aldopentoses (xylose and particularly arabinose) were more reactive toward the MR
681 than ketosis (fructose) and aldohexoses (glucose), allowing higher rates of crosslinking,
682 resulting in such peculiar microstructural arrangements. These film-forming ultra-structures are
683 in agreement with FT-IR analysis, confirming the above observed results, in terms of thermal,
684 mechanical and barrier behaviors (Mujeeb Rahman *et al.*, 2018; Rubentheren *et al.*, 2016).

685 **3.7. Antioxidant activities of Cs-based MR crosslinked films**

686 The global antioxidative mechanism could be ascribed to the cooperative effects of
687 different systems, such as metal chelation and radicals scavenging (Zhang, Chen, Zhang, Ma
688 and Xu, 2013). Therefore, to investigate the antioxidativity of the Cs-based MR crosslinked
689 films, different *in vitro* antioxidant tests were implemented: DPPH radical scavenging (free
690 radicals quenching), reducing power (iron reduction ability) and metal chelating assays (**Fig.**
691 **6**). Data in the present work reveal that for all antioxidant systems investigated, values were
692 remarkably higher ($p < 0.05$) in Cs-based MR crosslinked films than in the blank Cs-based film.
693 Indeed, blank Cs-based film exhibited weak reducing power of $OD_{700} = 0,165 \pm 0009$ (**Fig. 6A**),
694 whereas, good antioxidant activities of $74.1 \pm 0.58\%$ and $75.27 \pm 1.91\%$ were displayed for
695 DPPH radical scavenging (**Fig. 6B**) and metal chelating (**Fig. 6C**). The availability of -OH and
696 -NH₂ groups throughout the polymer matrix reflected the antioxidant ability of Cs, thus Cs-
697 based films antioxidant patterns (Badano *et al.*, 2019; Hamdi *et al.*, 2019a).

698 Subsequently to the MR crosslinking and the incorporation of saccharides into the film-
699 forming matrix, the antioxidant activity was enhanced and values increased and was directly
700 correlated to the amount of added saccharide. Interestingly, the reducing power antioxidant
701 activity of Cs-based MR treated film with 0.5% and 2.0% of arabinose (w/w Cs) increased by
702 more than 13- and 16-folds, respectively, compared to the blank Cs-based film (**Fig. 6A**).
703 Moreover, the highest values of inhibition of 100% for DPPH radical scavenging (**Fig. 6B**) and
704 metal chelating (**Fig. 6C**) were exhibited, at the same concentration of arabinose. Several
705 mechanisms could be implicated in the antioxidant activity of Cs-based MR-treated films.
706 Indeed, the reducing power could be attributed either to MR products formed in the primary
707 phase of the MR during thermolysis of Amadori compounds, or to MR final stage heterocyclic
708 compounds (Chen *et al.*, 2019). Furthermore, the marked increase in the scavenging activity of
709 MR-treated films could be associated with the reduction of the number of reactive amino groups
710 produced by the MR, otherwise, the intermediates and the final brown polymer (melanoidins)
711 could behave as hydrogen donors (Akar, Küçük and Doğan, 2017; Zhang, Shen, Zhu and Xu,
712 2015).

713 In line with above-mentioned in the FT-IR analysis and perceived by SEM, these findings
714 could explain the fact that the extent of the MR, as reflected by higher in vitro antioxidant
715 activities, was influenced by the type of saccharides ($p < 0.05$). Pentoses (arabinose and xylose),
716 with less carbons in their macromolecular chain, showed higher reducing power (**Fig. 6A**),
717 radical scavenging (**Fig. 6B**) and metal chelating activities (**Fig. 6C**), compared to the hexoses
718 (fructose and glucose), with more carbons. More particularly, aldoses (arabinose, xylose and
719 glucose), with a carbonyl group located at one end of the carbon chain, were more reactive than
720 ketosis (fructose), with a carbonyl group situated inside the carbon chain, which could be the
721 result of higher covalent crosslinking extent.



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724

725 **Figure 6:** Antioxidant activities of Cs-based films conjugated with different saccharides, at
 726 different mass ratios (0.5, 1.0 and 2.0 %; w/w Cs), through MR at 90 °C for 24 h, based on the
 727 reducing power (A), DPPH free radical scavenging (B) and metal chelation (C) *in vitro* tests.
 728 Cs: Chitosan, A: Arabinose, F: Fructose, G: Glucose, X: Xylose. Different letters (a-c) in the same films group
 729 are significantly different as determined by ANOVA test (p<0.05). Different letters (A-D) indicated significant
 730 differences between different films groups (p<0.05).

731 **4. Conclusion**

732 In the present work, different types of saccharides were incorporated at different mass
733 ratios into chitosan film forming solution. Maillard reaction crosslinking was induced by films
734 heating at 90 °C during 24 h. Advantageously, Maillard reaction increased water barrier and
735 resistance of resulted films, as reflected by water solubility, water contact angle and water vapor
736 permeability, in a saccharide type and dose-dependent manner. Treated films crosslinked with
737 aldopentoses were, additionally, less transparent with enhanced light barrier than ketoses-
738 treated films. Moreover, Maillard reaction allowed an improvement of the thermal resistance
739 (in terms of glass transition and degradation temperatures) and mechanical behavior, compared
740 to blank non-treated film. Furthermore, Maillard reactions could be considered as an alternative
741 tool to develop chitosan-based films with improved antioxidativity that could then be used as
742 active packaging against foods oxidation, especially using aldopentoses as crosslinkers. The
743 subsequent application of the prepared films in the packaging of food samples, such as oil, fish
744 fillet, etc., is of great interest to concretely confirm their effectiveness.

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Supplementary data

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