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Vargas, M.; Albors, A.; Chiralt A.; González Martínez, MC. (2011). Water interactions and microstructure of chitosan-methylcellulose composite films as affected by ionic concentration. *LWT - Food Science and Technology*. 44(10):2290-2295.
doi:10.1016/j.lwt.2011.02.018



The final publication is available at

<http://doi.org/10.1016/j.lwt.2011.02.018>

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Additional Information

1 **Water interactions and microstructure of chitosan-**
2 **methylcellulose composite films as affected by ionic**
3 **concentration**

4 María Vargas (*), Ana Albors, Amparo Chiralt, Chelo González-Martínez
5 Departamento de Tecnología de Alimentos – Instituto Universitario de Ingeniería de Alimentos
6 para el Desarrollo
7 Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.
8 (*) *Telephone:* 0034 96 387 70 00 Ext. 73642, *Fax:* 0034 96 387 73 69, *e-mail:* mavarco@tal.upv.es

9
10
11 **Abstract**

12 Edible films based on high molecular weight chitosan (CH) and
13 methylcellulose (MC) were obtained by mixing different ratios (0:1, 0.5:1.5,
14 1.0:1.0, 1.5:0.5 and 1:0) of the biopolymers in two solvent conditions (0.95 and
15 6.85 mmol of ions per g polymer). In order to characterize the dry films, water
16 sorption isotherms, water vapour permeability and film microstructure were
17 evaluated. Water vapour permeability of CH-MC composite films were
18 significantly affected by both the CH-MC ratio and the ionic concentration in
19 the matrix. This can be attributed to the influence of ions on polymer chain
20 packaging during the film formation and their role in the water uptake capacity
21 of the films which affects the water transport properties.

22
23 *Keywords:* polysaccharides, film, coating, water vapour permeability, water sorption, microstructure

24

25

26 **1. Introduction**

27 Chitosan is a biodegradable cationic polymer with antimicrobial activity
28 (Cuero, 1999; Jung & Kim, 1999; No, Park, Lee, & Meyers, 2001;
29 Tharanathan & Kittur, 2003; Zheng & Zhu, 2003) and film-forming ability (Li,
30 Dunn, Grandmaison, & Goosen, 1992). However, the uses of chitosan based
31 films are limited because of their high water vapour permeability (Butler,
32 Vergano, Testin, Bunn, & Wiles 1996; Caner, Vergano, & Wiles, 1998) that
33 can be improved by combining chitosan with other materials that soften its
34 hydrophilic character (Park, Marsh, & Rhim, 2002; Vargas, Albors, Chiralt, &
35 González-Martínez, 2009; Xu, Kim, Hanna, & Nag, 2004). To this end,
36 Hoagland and Parris (1996) developed chitosan/pectin laminated films by
37 interacting cationic groups of chitosan with the anionic groups of pectin. Xu et
38 al. (2004), observed a decrease in water vapour transmission rates by
39 combining chitosan with two thermally gelatinized corn starches. Pinotti,
40 Garcia, Martino, and Zaritzky, (2007) studied the mechanical properties and
41 the microstructure of chitosan (CH) based films as affected by methylcellulose
42 content (MC) and García, Pinotti, Martino, and Zaritzky, (2004) evaluated the
43 water vapour permeability of these CH-MC composite films, which had
44 intermediate permeability values compared to the films of pure components.
45 However, to the best of our knowledge, there are no reported studies into the
46 water sorption isotherms of such films and their relationship with their water
47 barrier properties and film microstructure, which in turn are affected by the
48 solvent conditions (ionic strength, pH, etc.) used to obtain the film forming
49 solutions of polymers.

50 The permeability of a film involves solubilisation and diffusion of water
51 molecules through the film matrix. Biopolymers such as chitosan and
52 methylcellulose are plasticized by water due to their inherent hydrophilic
53 nature. The water vapour uptake of hydrocolloid films depends on both the
54 chemical structure of the components and on their morphology. This is
55 particularly significant for chitosan, since its conformation in solution greatly
56 depends not only on structural parameters like the degree of acetylation and
57 chain length but also on solution parameters, such as ionic strength, solvent,
58 temperature, pH, dielectric constant of the solvent, etc. (Sorlier, Viton, &
59 Domard, 2002). In this sense, Thongngam and McClements (2005) showed
60 that the addition of NaCl to chitosan-sodium taurocholate systems weakened
61 the electrostatic interactions between the bile acid and the chitosan, thereby
62 reducing the driving force and favouring polymer binding.

63 The purposes of this study are to obtain composite edible films based on
64 chitosan and methylcellulose and to evaluate the effect of the ratio of both
65 polymers and the solvent conditions to obtain the film forming solutions on the
66 water sorption isotherms, microstructure and water vapour permeability of the
67 dry films.

68

69 **2. Materials and methods**

70 *2.1. Materials*

71 High molecular weight chitosan (CH), with a deacetylation degree of 82.7%
72 (CAS Number 9012-76-4, Sigma-Aldrich, Batch 14624DB, Brookfield
73 viscosity of 919 mPa·s at 1 g/ 100 g in 1 g /100 g acetic acid solution);
74 Methylcellulose, MC (50.6 KDa, Methocel A15 Food Grade, degree of

75 substitution: 1.8, Dow Chemical Company, Midland, USA), 98% glacial acetic
76 acid, NaOH, and Tween 80 (Panreac, Barcelona, Spain), were used to obtain
77 the film-forming solutions (FFSs).

78

79 *2.2. Preparation of film-forming solutions*

80 Chitosan (2 g/100 g) was dispersed in an aqueous solution of glacial acetic acid
81 (1 mL/100 g), and the pH was adjusted with NaOH (2 mol·L⁻¹) to 3.8 (solvent
82 I) or 5.2 (solvent II). Methylcellulose (2 g/100 g) was dispersed in a sodium
83 acetate buffer solution (175 mmol·L⁻¹) at pH 3.8 or 5.2. Concentration of
84 buffer was calculated to obtain a similar ionic strength to that of chitosan
85 solutions. Chitosan and methylcellulose solutions were mixed in different
86 proportions to obtain the following CH-MC ratios: 0:1, 0.5:1.5, 1.0:1.0, 1.5:0.5
87 and 1:0. The ratio ions-polymer in solvent I and solvent II were 0.95 and 6.85
88 mmol/g polymer, respectively. Tween 80 at 0.1 mL/100 g was added to the
89 aqueous solutions to improve wettability. A pH-meter C831 (Consort,
90 Tumahout, Belgium) was used to determine the pH of the FFSs at 25 °C.

91

92 *2.3. Preparation of films*

93 Films were obtained by casting. FFSs were poured into a framed and levelled
94 polytetrafluorethylene plate ($\phi = 15$ cm) and were dried at room temperature
95 for 48 hours. Films were prepared by pouring the amount of FFS that will
96 provide a surface density of polymer in the dry films of 56 g/m². Dry films
97 were peeled off from the casting surface and preconditioned in desiccators at
98 5°C and 58.5 % RH prior to testing.

99

100 2.4. *Film thickness*

101 Film thickness was measured using a hand-held digital micrometer (Palmer -
102 Comecta, Spain, ± 0.001 mm). The thickness of each film was measured five
103 times and the mean values were used in water vapour permeability
104 calculations.

105

106 2.5. *Water sorption isotherms*

107 For the adsorption experiments, film pieces of about 3 cm in diameter were
108 transferred to vacuum chambers containing P_2O_5 to complete drying.
109 Afterwards, film specimens, in triplicate, were placed at 5 °C in hermetic
110 chambers containing oversaturated salt solutions with different water activity
111 (a_w): LiCl (0.11), $MgCl_2$ (0.34), K_2CO_3 (0.43), $Mg(NO_3)_2$ (0.59), NaBr (0.64),
112 KI: (0.73), NaCl: (0.76), KCl (0.88). Samples were weighed periodically till a
113 constant value ($\Delta m \approx 0.0005$ g) was reached, where the equilibrium was
114 assumed to be achieved (Spiess & Wolf, 1983). Finally, the equilibrium
115 moisture content was determined using a vacuum oven at 70 °C for 24 h.
116 Moisture sorption isotherms (equilibrium moisture content dry basis vs water
117 activity) were fitted by means of BET and GAB models (Eq. 1 and 2) and
118 model parameters were obtained.

119
$$\frac{a_w}{(1-a_w) \cdot W_e} = \frac{1}{W_0 \cdot C} + \frac{C-1}{W_0 \cdot C} \cdot a_w \quad (\text{Eq. 1})$$

120
$$\frac{a_w}{W_e} = \frac{1}{W_0 \cdot C \cdot K} + \frac{C-2}{W_0 \cdot C} \cdot a_w + \frac{K \cdot (1-C)}{W_0 \cdot C} \cdot a_w^2 \quad (\text{Eq. 2})$$

121 Where,

122 W_e equilibrium moisture content on a dry basis (g water/g dry film)

123 W_0 adsorbed monolayer moisture content (g water/g dry film)

124 C constant related to thermal effects

125 K factor related to the total heat of sorption of the multilayer

126 a_w water activity

127

128 2.6. Water vapour permeability

129 Samples of dry films ($\phi = 7$ cm) were stored in desiccators at 58.5% relative
130 humidity (RH) and 5 °C for at least 15 days prior to the evaluation of the water
131 vapour transmission rate. Water vapour permeability (WVP) was measured, in
132 quintuplicate, according to the “water method” of the ASTM E-96-95 (ASTM,
133 1995), using polymethylmethacrylate cups following the design proposed by
134 Gennadios, Weller, and Gooding (1994). Deionised water was used inside the
135 testing cup to achieve 100 % RH on one side of the film, while an
136 oversaturated magnesium nitrate solution was used to control the relative
137 humidity on the other side of the film: 58.5 %. During WVP testing, the side of
138 the film in contact with the PTFE plate was placed in contact with the part of
139 the test cup having the highest RH. This situation tries to simulate the case of a
140 film applied on the wet surface of a fresh cut vegetable or fruit. A fan placed
141 on the top of the cup was used to promote convection. Water vapour
142 transmission rate measurements (WVTR) were performed at 5 °C to reproduce
143 the storage conditions of fresh-cut coated products. To calculate WVTR (Eq.
144 3), the slopes of the steady state period of the curves of weight loss as a
145 function of time were determined by linear regression. For each type of film,
146 WVP measurements (Eq. 4) were carried out in triplicate.

$$147 \text{ WVTR} = \frac{J}{A} \quad (\text{Eq. 3})$$

148
$$\text{WVP} = \frac{\text{WVTR}}{P_{w1} - P_{w2}} \cdot L \quad (\text{Eq. 4})$$

149 Where

150 WVTR water vapour transmission rate, $\text{g} \cdot \text{h}^{-1} \cdot \text{m}^{-2}$

151 J slope of the plotting of weight loss vs. time, g h^{-1} .

152 A area of the film, m^2

153 P_{w1} partial pressure of water vapour on the film's underside, Pa

154 P_{w2} partial pressure of water on the film's upper surface, Pa

155 L film thickness, m

156

157 *2.7. Scanning electron microscopy*

158 Dry films were stored in a desiccator with P_2O_5 for at least 15 days.

159 Microstructural analysis of cross-sections of the films was carried out using

160 SEM technique in a JEOL JSM-5410 (Japan) electron microscope. Pieces of 5

161 x 1 mm were cut from films and mounted in copper stubs. Samples were gold

162 coated and observed using an accelerating voltage of 10 kV.

163

164 *2.8. Statistical analysis*

165 Results were analysed by multifactor analysis of variance with 95%

166 significance level using Statgraphics ®Plus 5.1. Multiple comparisons were

167 performed through 95% Least Significant Differences (LSD) intervals.

168

169 **3. Results and discussion**

170 *3.1. Water sorption isotherms*

171 The water sorption isotherms (WSI) at 5 °C of pure CH and MC films together

172 with the composite films prepared by mixing CH and MC at different

173 proportions and conditions are shown in Figures 1a-d. The WSI of the films
174 obtained with solvent I (pH=3.8) conditions were sigmoid in shape, increasing
175 slowly in line with a_w up to 0.45, beyond which a steep rise in moisture content
176 was observed, owing to the solubilisation phenomenon. This sigmoid shape
177 was also observed by other authors in pure CH films (Del Nobile, Buonocore,
178 & Conte, 2004; Wiles, Vergano, Bunn, & Testin, 2000). According to
179 Fernández Cervera et al. (2004) CH has three predominant adsorption sites:
180 hydroxypropyl group, amine group and polymer chain end (composed of a
181 hydroxyl group or aldehyde group).

182 For films obtained by using solvent II (pH=5.2), a change in the sorption
183 behaviour of the matrix was observed and the shapes of the isotherms were
184 more typical of those systems rich in low molecular weight or ionic
185 compounds, such as salts and sugars (Okos, 1985), and exhibited a greater
186 equilibrium moisture content for intermediate and high water activity values,
187 whereas at low water activity, the opposite trend was observed. This change in
188 the WSI could be explained by taking into account the sorption effect of the
189 greater amount of ions per g of polymer, which were incorporated to fit the pH
190 of the FFSs at 5.2. The presence of ions increases the water sorption capacity
191 of the matrix at intermediate and high a_w levels, as ions interact with water
192 molecules and solubilisation phenomenon occur to a greater extent in low
193 molecular charged species. At low water activity, the predominant adsorption
194 on the active sites of the polymer network occurs and, in this sense, the more
195 neutral character of the CH chains in solvent II (pH near isoelectric point) can
196 explain the lower equilibrium water content in this case.

197 For pure films from solvent I, the CH films presented the highest water
198 sorption capacity due to their greater hygroscopic nature. In solvent II, the
199 opposite trend was observed due to the greater amount of ions added and their
200 particular interactions with the polymer chains. Whereas the acetate groups are
201 partially neutralised by the positive groups of the CH molecules, this
202 neutralization does not occur in the non-ionic chain of MC. Therefore, pure
203 MC from solvent II presented the greatest water adsorption capacity, due to the
204 presence of sodium and acetate ions, which are non-neutralized with polymer
205 chain groups and so more available to interact with water molecules. The effect
206 of charged groups in the polymer chains and the counterions has been studied
207 by some authors (Berthold, Desbrières, Rinaudo, & Salmén, 1994; Jouon,
208 Rinaudo, Milas, & Desbrières, 1995). A notable effect of dissociation degree of
209 the chain groups and type of counterions was observed on the water sorption
210 behaviour. For uncharged polymers such as CMC only tightly interacting water
211 molecules are adsorbed and non-freezing water was detected at the equilibrium
212 water contents in the complete RH range, which indicate the scarce mobility of
213 the adsorbed water. Nevertheless, for charged polymers such as CH, freezing
214 water was detected for equilibrium moisture contents corresponding to relative
215 humidity below 100%, thus indicating that a fraction of water molecules are
216 bonded to the ionic groups showing greater mobility. Water mobility degree
217 will affect the WVP of the films.

218 Experimental sorption data were fitted to GAB and BET models and the results
219 are reported in Table 1. To fit the GAB model, the second-degree polynomial
220 equation was used for the regression analysis (Eq. 2). Due to the high degree of
221 mathematical correlation among the three GAB parameters (Schär & Rüegg,

222 1985) their physical meaning is not considered, although the model predicted
223 values are used to plot water sorption isotherms (Figure 1) over the whole a_w
224 range. On the other hand, the BET equation constants (obtained for data with
225 $a_w < 0.6$), which have a thermodynamic base, were used to interpret the
226 interaction between the components with water molecules. Nevertheless, the
227 fitting of BET model to the isotherm curves did not give coherent values of the
228 parameters in solvent II, due to the hyperbolic shape of the isotherms, and so,
229 they were not considered in this case. The monolayer moisture contents of the
230 CH pure film from solvent I were higher than the values reported in the
231 literature (Fernández Cervera et al., 2004), which can be attributed to the
232 different temperature at which these values were determined (20 °C) and the
233 different nature of the chitosan used (degree of acetylation and molecular
234 weight). For CH-MC composite films obtained in solvent I BET parameters,
235 W_0 and C , increased when the CH content increased, due to the greater
236 hygroscopic nature of this polymer and to the stronger interactions established
237 between water molecules and the substrate at low a_w values, where no
238 solubilisation phenomenon occur.

239 In order to analyse interactions between MC and CH in the composite films,
240 the equilibrium moisture content of these films at each a_w was compared with
241 the value obtained by applying Eq. 5. In this equation, W_e was obtained from
242 the equilibrium value of pure CH and MC films and its ratio in the dried film.

243

$$244 \quad W_{\text{est}} \Big|_{a_w} = X_{\text{CH}} \cdot W_{\text{eCH}} + X_{\text{MC}} \cdot W_{\text{eMC}} \quad (\text{Eq. 5})$$

245 Where,

246 $W_{\text{est}} |_{a_w}$ equilibrium moisture content of the composite film (d.b.) at a constant
247 a_w .

248 X_{CH} mass fraction of chitosan in the composite dry film.

249 X_{MC} mass fraction of methylcellulose in the composite dry film.

250 W_{eCH} equilibrium moisture content of pure chitosan film (d.b) at a constant a_w .

251 W_{eMC} equilibrium moisture content of pure methylcellulose film (d.b) at a
252 constant a_w .

253 Figure 2 shows experimental versus predicted equilibrium moisture content for
254 CH-MC composite films. For solvent I, predicted and experimental W_e values
255 for composite films are located very close to the diagonal, although
256 experimental values are slightly greater than predicted, which can be explained
257 by the adsorption effects of the small amounts of ions. This seems to indicate
258 that no interactions between film components could be assumed and that they
259 are mostly in separate phases, as the observed behaviour can be explained by
260 taking only into account the sorption behaviour of pure polymers and their
261 mass fraction in the mixture.

262 When using solvent II, experimental values are greater than the predicted ones,
263 especially for the film containing a greater proportion of CH. This indicates
264 that the ions present play an important role in the water adsorption capacity of
265 the film, modifying the molecular interactions in the matrix. In this sense, it is
266 important to point out that the ratio between the amount of ions and the amount
267 of chitosan changes in the different polymer mixtures, which can play an
268 important role in the sorption behaviour of the composite.

269

270 *3.2. Water vapour permeability*

271 The relative humidity conditions used for the measurement of water vapour
272 permeability (100/59) of the films were established to simulate the
273 environmental conditions when the films are applied as a coating for food
274 stored at 5°C. The average thickness of the films and standard deviation (σ)
275 was 71 μm ($\sigma = 25$) for solvent I conditions and 127 μm ($\sigma = 29$) for films
276 prepared with solvent II. Likewise, there was an observed tendency of the film
277 thickness to increase in line with a greater ratio of CH in the matrix. The
278 increase in the film thickness indicates that the packaging of polymer chains in
279 the dried films is less compact when ionic concentration is greater due to the
280 more folded macromolecular conformation in the initial solution, which made
281 the chain aggregation more difficult when solvent is eliminated.

282 As seen in Figure 3, films obtained by using solvent II presented higher WVP
283 than those obtained with solvent I. As can be seen in the sorption isotherm
284 curves, the water content of solvent II films is greater than that of solvent I
285 films, at the relative humidity values corresponding to the WVP experiments
286 (0.59-1.0 a_w range). This higher water content will plasticise the film structure,
287 and, as commented above, water bonded to ions or ionic groups have greater
288 mobility (freezing water). These factors favour water molecule mobility and
289 diffusion, thus contributing to increase WVP. Likewise, the ions hinder the
290 polymer chain packaging due to their conformational effects in solution, giving
291 rise to more folded tails which affects their arrangement in solid phase when
292 the solvent is evaporated and which leads to more open microstructures, where
293 the water transport is made easier. The greater film thickness obtained for
294 solvent II films coincides with this effect. The promotion of WVP by the
295 increase of the ionic concentration in the film is especially marked for pure MC

296 films, probably due to the neutral character of the polymer which does not
297 favour the bonding and adsorption of the ions on the chain, which are freer in
298 the solvent of the FFSs, thus having a greater effect on the molecular
299 conformation. In this sense, it is remarkable that the ionic concentration did not
300 affect WVP to so great an extent in pure CH films which can be explained by
301 the tight link and adsorption of ions on the chain's charged groups and their
302 more limited availability in the solvent. In the CH-MC films with higher ionic
303 concentration, the WVP decreased as the CH increased in the mixture, in
304 agreement with the above-mentioned effect. The WVP values for pure CH
305 films at the 100/59 RH gradient were in the range of those reported by Park
306 and Zhao (2004) and Vargas et al. (2009).

307

308 *3.3. Microstructure*

309 Figure 4 shows the SEM micrographs of composite films obtained from
310 solvents I and II. As has been commented above, from the WVP data, films
311 prepared with solvent I show a more compact multilayered structure where a
312 more regular packaging of polymer chains can be observed. Nevertheless, in
313 films prepared with solvent II a greater degree of polymer chain folding occurs
314 which hinders the regular and lineal aggregation of the polymer chains. This
315 leads to a more open matrix, which facilitates the water transport.

316

317 **4. Conclusion**

318 Water vapour permeability and water sorption capacity of CH-MC composite
319 films were significantly affected by both the CH-MC ratio and the ionic
320 concentration in the matrix. This is explained by the influence of ions on the

321 macromolecular conformation in the FFSs and its effect on the polymer chain
322 packaging during the film formation. Likewise, the ions also play an important
323 role in the water uptake capacity of the film at intermediate and high a_w values,
324 which contributes to a differing degree of matrix plasticization, thus affecting
325 the water molecule mobility and consequently the water transport properties.

326

327 **Acknowledgements**

328 The authors acknowledge the financial support provided by Ministerio de
329 Educación y Ciencia (Spain) for the Project AGL2007-65503. The authors also
330 acknowledge the Electronic Microscopy Service of Universidad Politécnica de
331 Valencia for their assistance in the use of SEM.

332

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416 with different molecular weights. *Carbohydrate Polymers*, 54, 527-530
417
418
419

420 **Table 1.** BET and GAB parameters obtained from isotherms of films
 421 formulated from pure components (CH: chitosan or MC: methylcellulose) and
 422 CH-MC mixtures.

Film	Solvent	BET Parameters		GAB Parameters		
		W_0	C	W_0	C	K
MC	I	0.075	6.2	0.0747	6.8	0.982
0.5CH-1.5MC		0.0777	31.6	0.0995	2070.9	0.865
CH-MC		0.0854	58.5	0.1058	445.5	0.877
1.5CH-0.5MC		0.1063	71.1	0.1285	54.6	0.875
CH		0.1249	350.7	0.1464	66.3	0.839
MC	II	-	-	0.28	3.35	0.95
0.5CH-1.5MC		-	-	0.0844	29.0	1.182
CH-MC		-	-	0.1469	6.1	1.094
1.5CH-0.5MC		-	-	0.3149	2.6	0.981
CH		-	-	0.1684	6.8	1.008

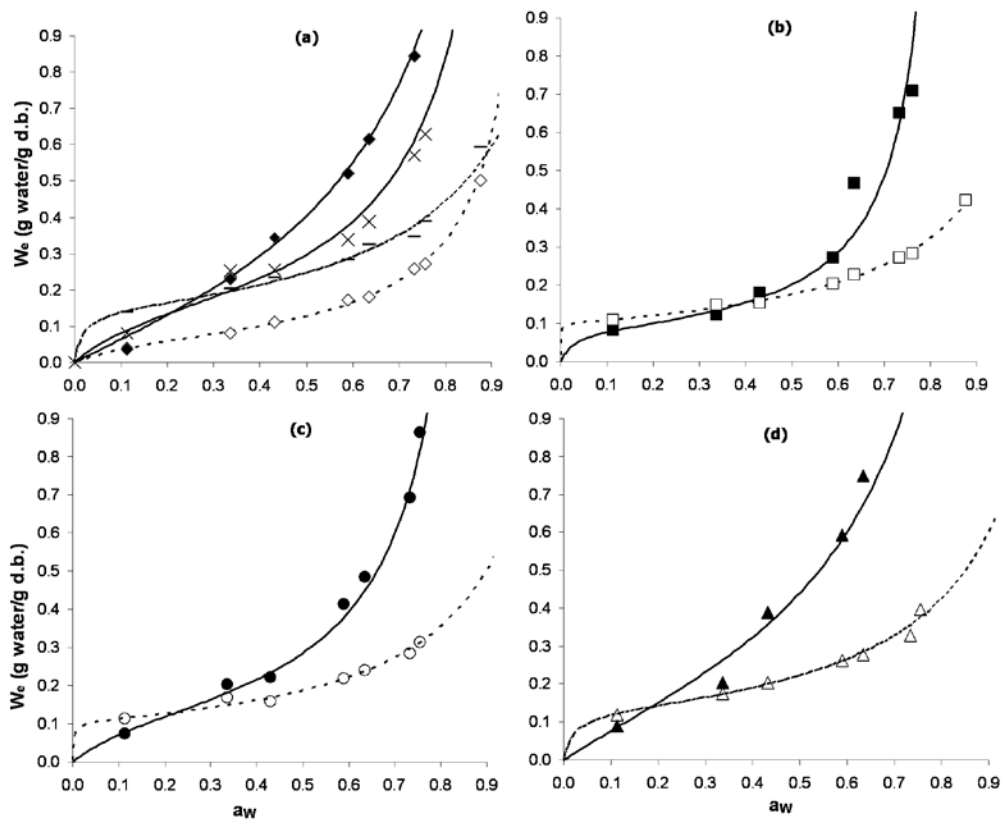
423 W_0 : (g water/g dry solids); C, K: constants related to the heat of sorption of the
 424 monolayer and the multilayer, respectively.

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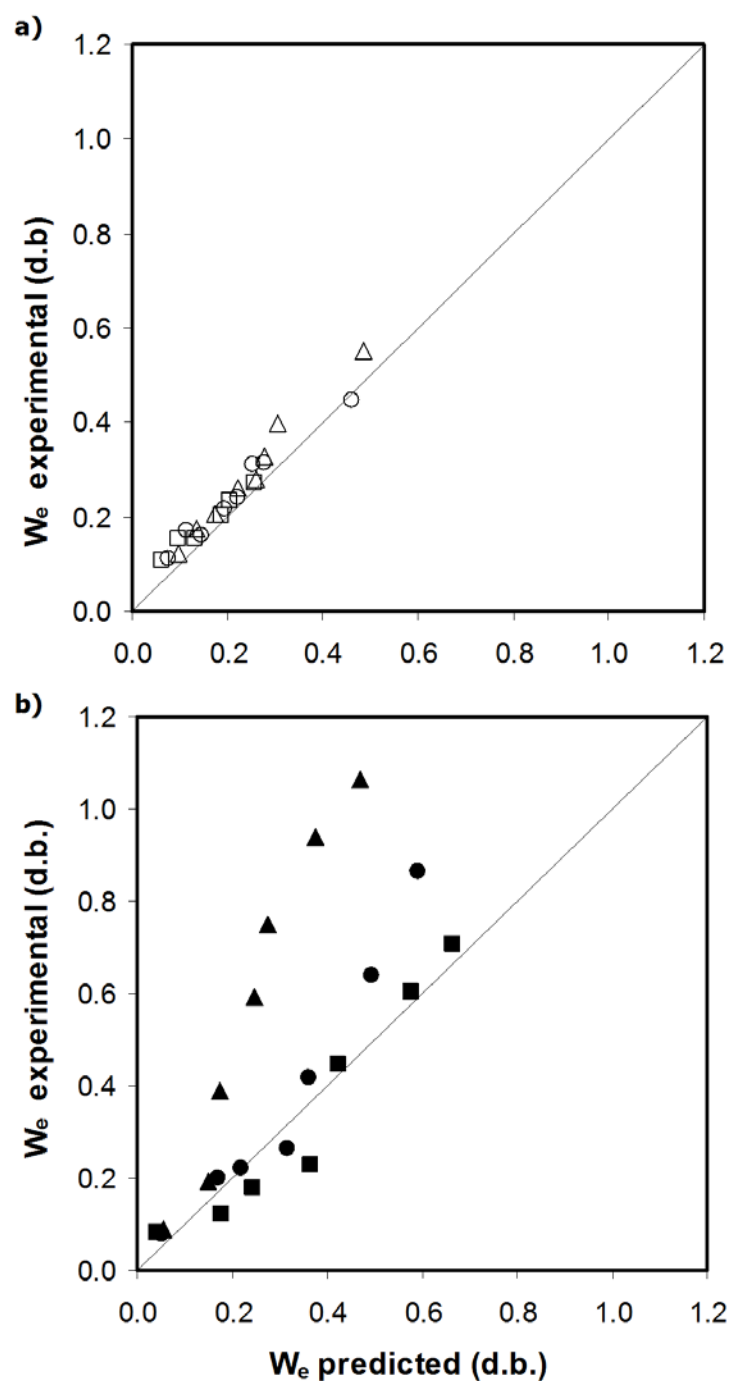


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430 **Figure 1.** Water sorption isotherms (experimental points and GAB fitted

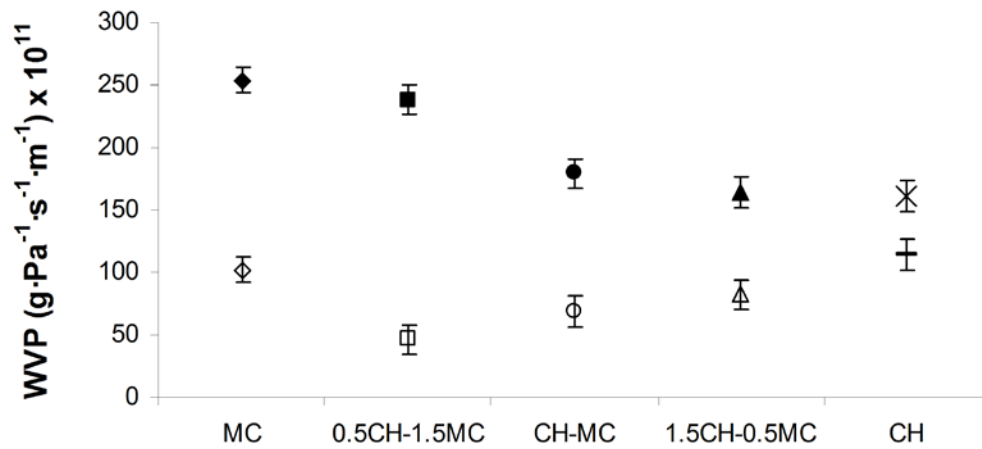
431 model) of pure films (a): \diamond MC solvent I, \blacklozenge MC solvent II, $-$ CH solvent I, \times

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434 **Figure 2.** Comparison between experimental values of equilibrium moisture
 435 content and those predicted by the linear model for the different composite
 436 films. a) \square 0.5CH-1.5MC, \circ 1.0CH-1.0MC, Δ 1.5CH-0.5 MC, solvent I (b)
 437 \blacksquare 0.5CH-1.5MC, \bullet 1.0CH-1.0MC, \blacktriangle 1.5CH-0.5MC, solvent II. CH:
 438 chitosan; MC: methylcellulose.



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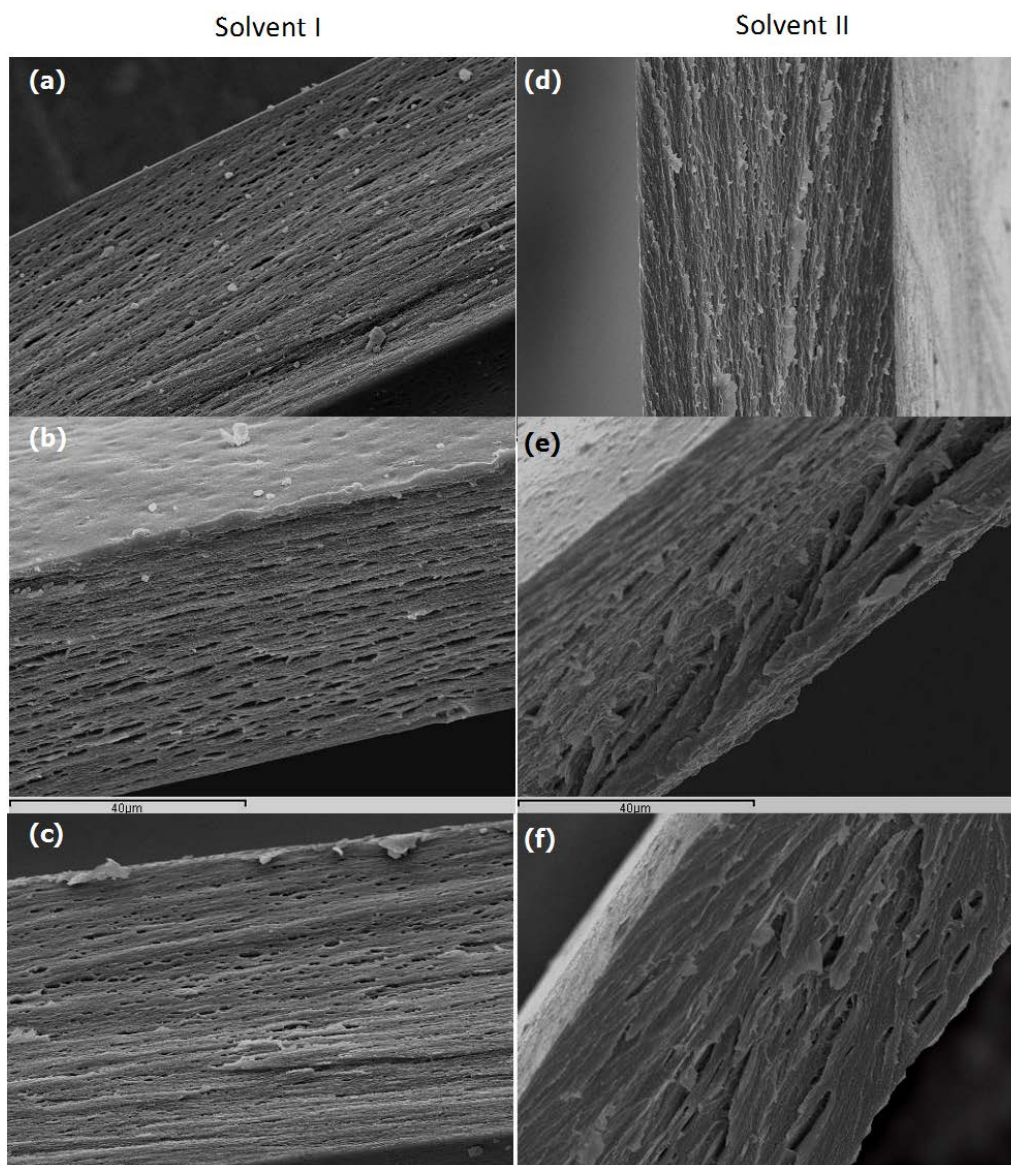
441 **Figure 3.** Water vapour permeability at 5 °C and 100/59 RH gradient of pure442 films (\diamond MC solvent I, \blacklozenge MC solvent II, $-$ CH solvent I, \times CH solvent II)

443 and CH-MC composite films obtained from solvent I (hollow symbols) and

444 solvent II (solid symbols). Mean values and 95 % LSD intervals. CH: chitosan;

445 MC: methylcellulose.

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Figure 4. SEM micrographs of the cross-sections of CH-MC composite films:

449 (a, d) 0.5CH-1.5MC; (b, e) 1.0CH-1.0MC (c, f) 1.5CH-0.5MC obtained from
450 solvents I (a,b,c) and II (d,e,f). Magnification is 1500x. CH: chitosan; MC:
451 methylcellulose.

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