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Additional Information

1 **Edible coatings controlling mass loss and *Penicillium roqueforti* growth**  
2 **during cheese ripening.**

3

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10 **Abstract**

11 The application of edible coatings carrying antifungal compounds on cheese  
12 was studied to reduce mass losses and control the fungal growth on the cheese  
13 surface during ripening. The effectiveness of 8 biopolymers and Aloe vera gel  
14 (AV) at controlling mass loss was analysed during the early stage of maturation,  
15 with and without lipids (Oleic acid and oleic acid-beeswax blend) and antifungal  
16 compounds (potassium sorbate (PS)), gallic tannin (GT) and Aloe vera gel. The  
17 gellan gum with both PS and GT exhibited the greatest efficacy at controlling  
18 the cheese water loss during the ripening period. The AV gel and its blend with  
19 gellan gum did not exert a good water vapour barrier capacity, although it did  
20 exhibit antifungal action against *Penicillium roqueforti*. The coating of gellan with  
21 PS resulted in an 84% inhibition of mycelial growth and could prevent fungal  
22 growth during cheese ripening, while controlling the cheese mass loss.

23 **Keywords:** Edible coatings, cheese, antifungal control, mass loss control,  
24 gellan gum, potassium sorbate.

25

26

## 27 **1. Introduction**

28

29 The amount of cheese consumed globally has increased over the years and the  
30 cheese industry is now an important sector of the economy. The superficial  
31 growth of fungi and yeast during the early stages of the ripening process  
32 represents one of the major losses for cheese manufacturers (*Costa et al.,*  
33 *2018*). Although these microorganisms proliferate on the surface, they can  
34 penetrate inside the cheese if cracks or some imperfections exist (*Var et al.,*  
35 *2006*), leading to significant product losses during the ripening step. On an  
36 industrial level, one of the most commonly used alternatives to mitigate this  
37 problem is the surface application of antifungals such as natamycin, also known  
38 as pimaricin (*González-Forte et al., 2019*). This active compound can be  
39 applied by spray systems or via an immersive process in a solution of polyvinyl  
40 acetate in order to form a coating that also helps to reduce water loss during the  
41 maturation process (*Sung et al., 2013; Thomas and Delves-Broughton, 2003*).  
42 Nevertheless, this coating makes the cheese surface inedible, while the  
43 antifungal migration to inner parts of the cheese (which varies according to the  
44 type of cheese) could constitute a risk for consumer health (*Var et al., 2006*).  
45 The use of edible polymers from renewable sources as cheese coating is a  
46 more adequate strategy, mainly for unripened cheese, in which the crust is yet  
47 not well-defined and is usually consumed.

48 Some food preservative agents, such as potassium sorbate, have been directly  
49 applied onto cheese after or during its formation with positive results, preventing  
50 the growth of moulds and yeasts. However, an undesired rapid compound  
51 diffusion within the cheese matrix was observed in these conditions. This cause  
52 a decrease of active compound concentration on the cheese surface, thus  
53 limiting its antimicrobial activity. Likewise, the organoleptic properties of the  
54 edible internal part of the cheese can be also affected by the compound  
55 diffusion (Costa et al., 2018; López et al., 2013).

56 Nowadays, antifungal compounds of natural origin are in increasing demand  
57 due to the fact that consumer associate them to healthier diets. Aloe vera gel is  
58 an incolorous mucilaginous, obtained from the fresh leaves of *Aloe* spp. that  
59 exhibit bioactive properties (Choi and Chung, 2003). Previous studies (*Ortega-*  
60 *Toro et al., 2017*) have proven the antifungal action of this gel against six fungi  
61 responsible for plant diseases. *Castillo et al. (2010)* also reported an inhibitory  
62 effect in the mycelial growth of *Penicillium digitatum*. These active properties,  
63 together with its filmogenic capacity, makes Aloe vera gel a potential candidate  
64 for the obtaining of active coatings, either by itself or combined with other edible  
65 film-forming polymers.

66 Likewise, tannins are a heterogeneous phenolic group of naturally occurring  
67 compounds with different structures that shares the capacity to sequester and  
68 precipitate proteins (*Guo et al., 2018*). The antimicrobial activity of tannins  
69 depends on both their chemical structure and microbial strains. Research into  
70 and the identification of tannins acting against specific microorganisms is a task  
71 in the early stages of development (*Huang et al., 2018*). Tannin antifungal

72 activity has been proven while inside a polymer matrix, such as gelatine (*Guo et*  
73 *al., 2018*).

74 Edible antifungal coatings with healthy active components could play an  
75 important role in the safety and quality of unripened and ripened cheese,  
76 making transport and storage easier, and diminishing fungal alterations while  
77 preventing water loss during ripening. Edible polymers can be carriers of  
78 different kinds of functional compounds, such as colorants, flavourers,  
79 antioxidants or antimicrobials (Tavassoli-Kafrani et al., 2016). Biopolymers,  
80 such as polysaccharides and proteins, or lipids, can be used to obtain edible  
81 films and coatings for cheese, carrying antifungal agents encapsulated in the  
82 polymer matrix that should limit the compound migration within the cheese  
83 matrix, making the antifungal action more effective on the cheese surface.  
84 Moreover, many of these compounds are biodegradable and biobased (coming  
85 from renewable sources), which contributes to reducing the environmental  
86 impact of the process, compared to the use of traditional cheese coatings.

87 In this context, the present study analyses the efficacy of 8 different  
88 biopolymers and Aloe vera gel applied as edible coatings on cheese samples at  
89 reducing the mass loss during the early stages of maturation. The effect of lipid  
90 and antifungal incorporation in this capacity was also analysed as well as the  
91 antifungal activity of the selected polymers carrying antifungal compounds.

92 The study deals with the reduction of losses in cheese production due to fungal  
93 deterioration, while controlling the weight losses of the product during ripening,  
94 by using edible coatings with antimicrobial agents obtained from renewable  
95 sources. All these aspects are framed in the concept of food engineering  
96 sustainability.

## 97 2. Materials and methods

98

### 99 2.1 Materials

100 Coating-forming systems were prepared with sodium alginate (viscosity: 5-40  
101 mPas,1%), methylcellulose (Mw: ~14kDa, viscosity: 15mPas), hydroxypropyl  
102 methylcellulose (Mw: ~86 kDa, viscosity: 2.6-5.6 mPas,2%) and sodium  
103 caseinate (Mw: ~23 kDa) provided by Sigma-Aldrich (St Louis, MO, USA), low  
104 acyl gellan gum (KELCOGEL F, CP Kelco Atlanta, GA, USA,  $M_w$   $3-5 \times 10^5$   
105 Da),  $\kappa$ -carrageenan (viscosity:5-25 mPas, 0.3%, Sosa ingredients, Barcelona,  
106 Spain), whey protein isolate Prodiet 90S (95% whey and 1.5% fat) from Ingredia  
107 (batch 131848, France) and xanthan gum (Mw  $\sim 10^6$  Da) (EPSA , Valencia,  
108 Spain). Glycerol (Panreac Química, Barcelona, Spain) was used as plasticizer  
109 when needed. Oleic acid (OA) and beeswax (BW) were used as lipids and  
110 supplied by Sigma-Aldrich (St Louis, MO, USA) and Fluka Analytical (Sigma-  
111 Aldrich Chemie GmbH, Steinheim, Germany) respectively. Potassium sorbate  
112 was obtained from Panreac Química (Barcelona, Spain). Commercial gallic  
113 tannin extract (Tanin Antiox White) was supplied by Dolmar (Haro, La Rioja,  
114 Spain), with 42 g GAE/100 g). Aloe vera gel was provided by the research  
115 group of Dr. Daniel Valero (UMH, Elche, Spain); the gel was directly extracted  
116 from *Aloe spp.* leaves and pasteurized at 75 °C. Natamycin (Sigma-Aldrich St  
117 Louis, MO, USA) was used as control in the assessment of the antifungal  
118 activity. Entrepinares (Valladolid, Spain) unripened pressed cheeses (1,05 kg),  
119 made with pasteurised cows' milk were purchased in a local market.

120

## 121 **2.2 Preparation of coating-forming systems (CFS)**

122 Aqueous coating-forming systems (CFS) were formulated using distilled water,  
123 by dissolving the maximum possible concentration of each polymer, previously  
124 determined, according to its solubility and film-forming ability, aiming to obtain  
125 the maximum solid density in the coating. The following amounts of polymer  
126 (g/100 of CFS) were used: Sodium alginate (ALG: 5.5), Gellan gum (GG: 0.9),  
127 Whey protein isolate (WPI: 8.0), k-Carragenan (kC: 0.6), Xanthan gum (XG:  
128 0.75), Hydroxypropyl methylcellulose (HPMC: 6.0), Methylcellulose (MC: 2.75),  
129 Sodium caseinate (SC: 15.0). Glycerol was added as plasticizer when required  
130 (WPI, XG and SC) at 0.3 g/g polymer. The CFS of all polymers were formulated  
131 without lipids and with 0.5 g lipid/ g polymer, using oleic acid (OA) or an oleic  
132 acid/ beeswax (OA/BW) mixture in a ratio of 7:3, according to (Fabra et al.,  
133 2008). All of the polymer solutions were prepared at 25°C under magnetic  
134 stirring, with the exception of WPI which was dissolved at 40 °C and  
135 subsequently heated at 90 °C for 30 min in order to promote molecular cross-  
136 linking (Lacroix et al., 2002). The lipids were incorporated into the polymer  
137 solution at 85 °C by homogenization with a rotor-stator (Ultraturrax Yellow Line  
138 DL 25 Basic, IKA, Staufen, Germany) for 1 min at 13,500 rpm and for 5 min at  
139 20,500rpm.

140 The antifungal agents, potassium sorbate (PS), Gallic Tannin (GT) or natamycin  
141 (NT), were incorporated into the selected CFS in the first experimental series on  
142 the basis of the best control of the cheese mass loss during ripening. The  
143 amount of each agent to be incorporated was determined to ensure a specific  
144 final content of antifungal in the dry coating. For PS, the amount used was 0.5  
145 mg/g cheese (lower than the legal limit in cheese according to *EU regulation*

146 #1129/2011). GT was incorporated to reach 1.5 mg/g cheese and the NT  
147 amount was 0.060 mg/dm<sup>2</sup> cheese according to the usual practices (CODEX  
148 STAN A-6, 1999). To determine the concentration of PS, GT and NT in the  
149 CFS, the amount of the CFS adhered per mass unit of cheese was taken into  
150 account to reach the target final concentration of antifungal agent in the cheese  
151 samples.

152 Aloe vera gel (AV) with 1% of soluble solids was directly applied to cheese  
153 samples as well as a blend of GG:AV with a 1:1 volume ratio. Samples were  
154 homogenized by magnetic stirring at 25 °C.

### 155 **2.3 Coating application on cheese samples and ripening control**

156 Cheese was purchased from the local market with a short maturation period (7-  
157 9 days) to simulate a fresh cheese matrix. For the mass loss studies, small  
158 cylinders from four different cheese pieces were obtained with approximate  
159 dimensions of 22 mm diameter and 24 mm height. All of the sample dimensions  
160 were measured individually in order to quantify the total sample surface area.  
161 Each treatment with the different CFS was applied in duplicate: two cylinders  
162 from two different cheeses from the same batch. Repetitions of some  
163 treatments were carried out with cheeses from different batches in two  
164 experimental series: the first included the coatings of different polymers with  
165 and without lipids and the second included the coatings of selected polymers  
166 with and without antifungal compounds.

167 A third experimental series was carried out to analyse the antifungal activity of  
168 the selected coatings. For the microbiological studies, 3 mm thick and 55 mm  
169 diameter slices of cheese samples were placed into a 55 mm Petri dish.



170 The coating were applied by sample immersion in the corresponding CFS for 1  
171 min, the samples were weighed before immersion and after draining the  
172 coating, as well as daily throughout storage (11 days) at 60% relative humidity  
173 (RH) and 4 °C. Uncoated control samples were also submitted to the same  
174 control.

175 For the microbiological study, a given CFS amount was poured over the cheese  
176 slice after it was placed into the Petri dish and allowed to dry for 24 h under  
177 60% RH and 4 °C. The CFS amount for each formulation was determined on  
178 the basis of the exposed sample area and the previously determined amount of  
179 CFS adhered per unit of surface area of the corresponding formulation in the  
180 immersion process.

181

## 182 **2.4 Fungal growth inhibition analyses**

183 *Penicillium roqueforti* was selected to carry out the antifungal tests due to the  
184 fact that it is a potential spoiler fungus in this kind of cheese and exhibited a fast  
185 growth (unpublished results), which permits a fast identification of the antifungal  
186 action of the studied coatings.

187 In order to analyse the growth inhibition capacity of the selected treatments,  
188 cheese samples in 55 mm Petri dishes were covered with 2.5 g of CFS,  
189 simulating the amount of CFS adhered in the immersive application. According  
190 to the printing method described by Sapper et al.(2018), the samples were  
191 inoculated by placing a 8 mm diameter disc of a 5-day growth potato dextrose  
192 agar (PDA) culture of *Penicillium roqueforti*. Afterwards, the samples were  
193 incubated at 25 °C for 7 days. Fungal growth was evaluated by measuring the

194 radial growth of the colonies in two perpendicular directions. The antifungal  
195 capacity of each treatment was evaluated through the mycelial growth inhibition  
196 (MGI) index calculated after 7 incubation days, using Eq. 1, where DC is the  
197 average expansion diameter in the control sample (inoculated uncoated cheese  
198 samples) and DO is the average growth diameter in each coated sample.

199

$$200 \quad \text{MGI} = \frac{DC-DO}{DC} \times 100 \quad (1)$$

201

## 202 **2.5 Statistical analysis**

203 A statistical analysis of data was performed through an analysis of variance  
204 (ANOVA) and regression analyses, using Statgraphics Centurion XVII software.  
205 Fisher's least significant difference was used at 95% confidence level.

206

## 207 **3. Results and discussion**

208

### 209 **3.1 Effectiveness of coatings at controlling mass loss.**

210 The effectiveness of the coating at controlling the mass loss of cheese samples  
211 will be affected by the coating thickness and the water vapour permeability of  
212 the formed coating. Taking into account the hydrophilic nature of most of the  
213 edible polymers used and the plasticising effect of the sample water, no great  
214 differences are expected between the permeability values of polymer coatings,  
215 although the film thickness should change according to the polymer  
216 concentration in the CFS and the amount of CFS adhered on the sample

217 surface. **Table 1** shows the mass of adhered CFS per mass unit of cheese after  
218 draining and the surface density of the adhered solids, estimated from the mass  
219 of adhered CFS, the solid mass fraction in the CFS and the sample surface  
220 area. Notable differences in the coating's adhered mass were found for the  
221 different polymer solutions, in line with the variation in the expected viscosity,  
222 which greatly affects the gravitational drainage after immersion (Marín et al.,  
223 2017). The highly viscous GG, kC and XG are the polymers that best promoted  
224 the adhesion of the CFS. In contrast, the greatest surface density of solids was  
225 reached in the SC coating, followed by the WPI and ALG, according to the  
226 highest solid content in the respective CFS. As expected, the incorporation of  
227 lipids into the polymer solutions modified both the retention of CFS, which  
228 showed greater variability, and the surface density of the solids, although these  
229 did not change the tendencies observed in the pure polymer's CFS. This can be  
230 attributed to the changes brought about by the CFS in both the cheese  
231 wettability and the CFS viscosity when lipids are present. The effect was  
232 different for OA and the OA/BW blend and depended on the polymer. A marked  
233 reduction in the solid surface density was observed for SC when OA was  
234 incorporated, whereas practically no effect was observed for the blend OA/BW.  
235 In WPI, both OA and OA/BW increased the solid surface density. However, OA  
236 reduced the adherence of CFS and surface density of solids in both HPMC and  
237 MC systems. In GG, the amount of adhered CFS decreased when OA was  
238 incorporated, although the surface density of solids was not negatively affected  
239 due to the higher solid content of CFS with lipids.

240 The highest amount of adhered solids (solid surface density values) was  
241 obtained for coatings obtained with SC with or without lipids, whereas the

242 lowest values were obtained for coatings from kC, XG, HPMC and MC. The  
 243 greater the surface density in the cheese surface, the higher the coating  
 244 thickness and the higher water vapour barrier capacity is expected. However,  
 245 potential interactions with the cheese surface could also affect the barrier  
 246 capacity of the coatings.

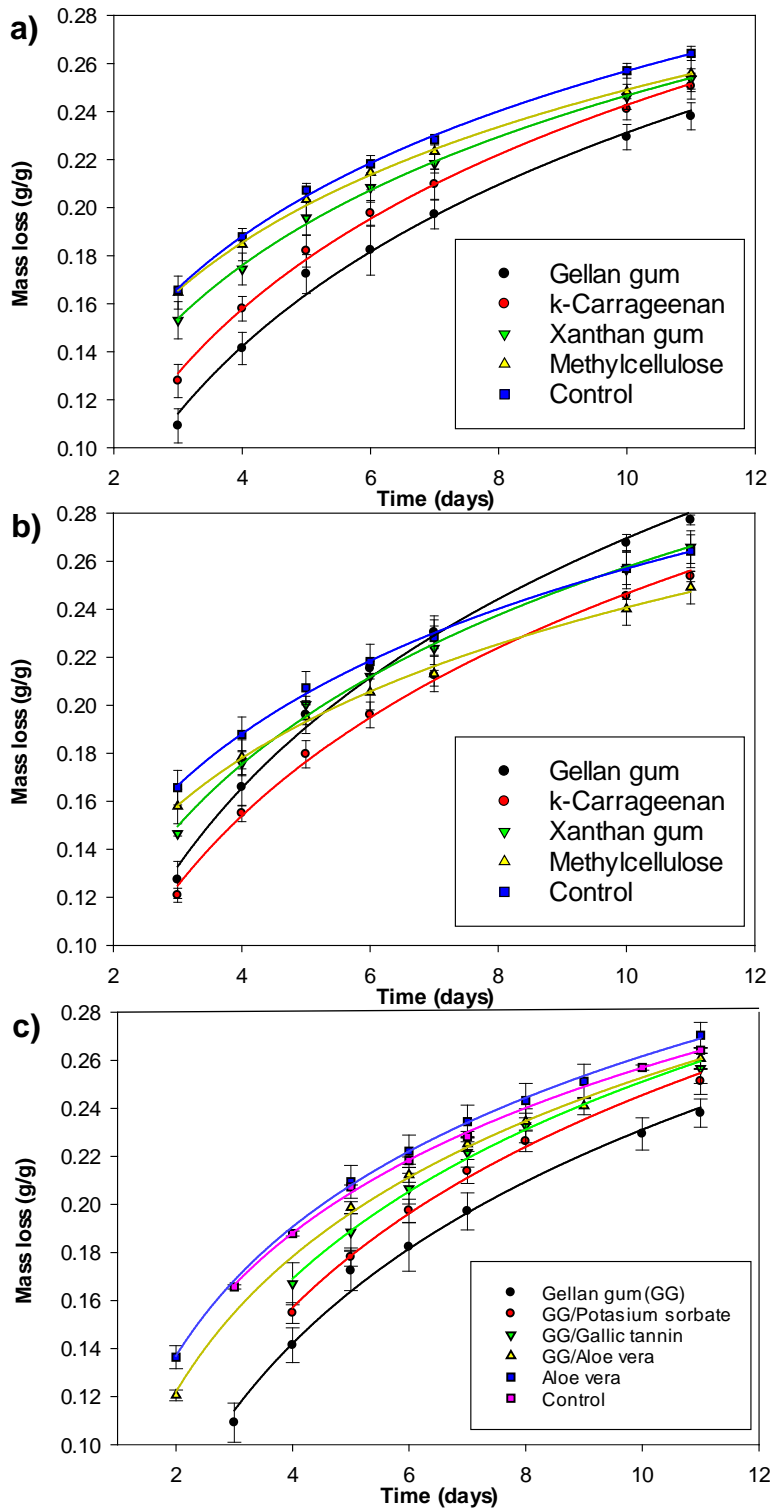
247 **Table 1.** Total adhered mass of coating-forming systems (CFS, g/g cheese) and surface density  
 248 of the coating solids (g/m<sup>2</sup>) (in brackets) for the different CFS containing, or not, lipids (oleic  
 249 acid: OA, or blends with beeswax (OA/BW). Variation coefficients for the adhered mass of CFS  
 250 were lower than: 10, 20 and 30 % for CFS without lipids and with OA and OA/BW, respectively.

Polymer	No lipids	With OA	With OA/BW
Alginate	0.64 (17)	0.54 (21)	0.59 (23)
Gellan gum	2.32 (9,3)	1.87 (13)	2.72 (17)
Whey protein	0.23 (11)	0.56 (38)	0.51 (31)
k-Carrageenan	2.06 (5,6)	2.04 (8,1)	2.93 (12)
Xanthan gum	1.04 (4,0)	1.14 (5,8)	1.34 (6,6)
HPMC	0.19 (5,4)	0.05 (2,2)	0.42 (15)
Methylcellulose	0.31 (4,0)	0.19 (3,8)	0.42 (7,3)
Caseinate	0.93 (86)	0.45 (56)	0.77 (92)

251

252 To analyze the effectiveness of the coatings at controlling water mass transfer  
 253 during the cheese ripening, the sample mass losses during storage was  
 254 quantified and the values are shown in **Figures 1a** and **1b** for some CFS  
 255 containing lipids or not. Similar behaviour was observed in all cases. A faster  
 256 mass loss occurred during the first 2-3 days of storage, when the coating crust  
 257 hasn't been formed yet and the polymer coating is highly plasticised by the  
 258 sample moisture. The mass loss rate (slope of the curves) decreased  
 259 throughout the storage time as the crust thickness grows and the surface water  
 260 content decreases, creating less plasticised coatings with better water vapour  
 261 barrier capacity. Coatings without lipids exhibited curves that are almost parallel

262 to the curve of the uncoated sample. Some coated samples (ALG, GG, kC, GX  
263 and SC) are above and others are below (WPI and HPMC) the control sample,  
264 reflecting the different barrier capacity of the coatings. GG without lipids  
265 exhibited the lowest mass loss values, while WPI presented the highest. Lipid  
266 incorporation modified the mass loss vs. time curves differently, depending on  
267 the polymer and the subsequent changes induced in the coating adhesion to  
268 the cheese. Nevertheless, in no case was the final mass loss of the samples  
269 (Figure 2) notably reduced with respect to the samples coated with the  
270 corresponding coatings without lipids, even in the SC treatment for which Fabra  
271 et al. (2009) reported an improvement in water vapour permeability when  
272 OA/BW was incorporated into sodium caseinate films. This suggests that, in the  
273 earlier ripening step where polymer coatings are highly plasticised, the water  
274 transfer rate was mainly controlled by the polymer matrix of the coating with  
275 only a slight effect of the dispersed lipid phase.



276

277 **Figure 1.** Mass loss (relative to the initial cheese mass) as a function of the time for coated and  
 278 uncoated cheese samples with different coating-forming systems. a) Only polymer coatings, b)  
 279 polymers with OA, c) Gellan gum with antifungal agents and Aloe vera.

280

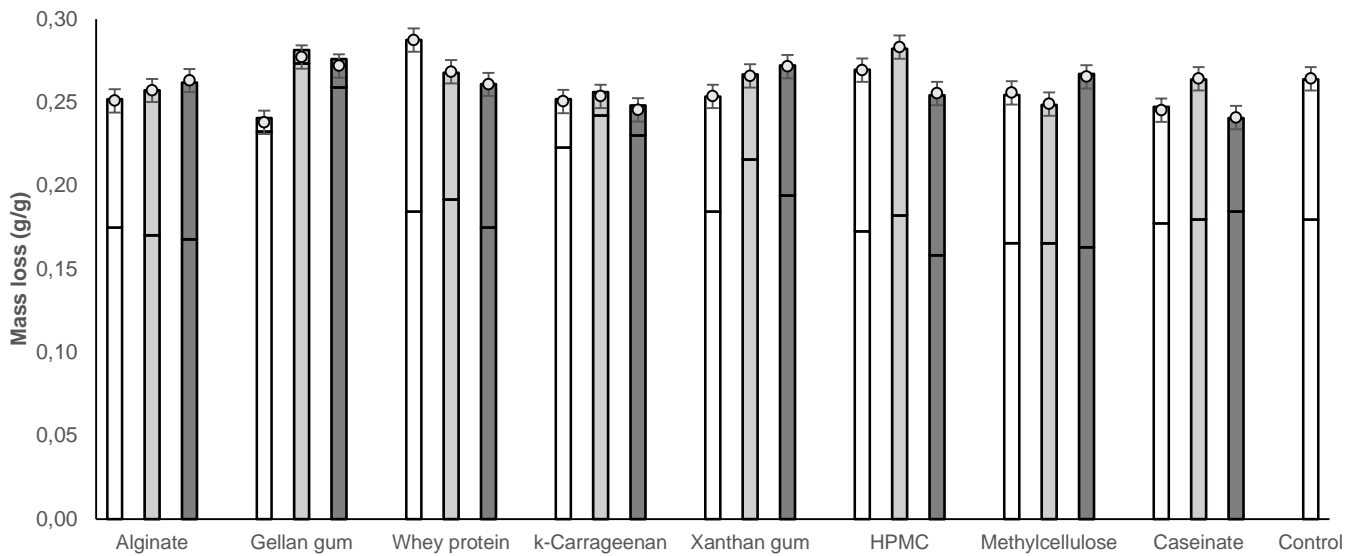
281 The mass loss behaviour during the short- term ripening process was well fitted  
282 ( $r^2 > 0.99$  in all cases) to an empirical equation **(2)**, where  $w$  corresponds to the  
283 percentage value of mass loss,  $t$  is time in days, while  $a$  and  $b$  are  
284 characteristic constants of a specific coating. The intercept  $b$  of the  
285 extrapolated curve represents a hypothetical fast mass loss occurring in the  
286 earlier ripening step, while slope  $a$ , in a logarithmic time scale, quantifies the  
287 mean mass loss rate during storage.

$$288 \quad w(t) = a \ln(t) + b \quad (2)$$

289 **Figure 2** shows the mass loss values obtained in the different samples after 11  
290 storage days in terms of the two components determined by the fitted equation:  
291 fast loss during the first days ( $b$  values) and slow loss at longer times ( $a \ln(11)$ ).  
292 The experimental values are also reflected (points) with the LSD values. The  
293 samples coated with GG presented the highest slow loss values ( $a \ln(11)$   
294 value), but the lowest fast loss ( $b$ ), which reflects the better ability of this  
295 polymer to control water loss at the beginning of the process, although it seems  
296 to delay the formation of the crust, thus allowing greater water loss in the rest of  
297 the analysed period. However, its overall effect on mass loss at the end of the  
298 period was positive, and samples exhibited the lowest total mass loss (23.8%  
299 *versus* 26.4% in the control sample). An attenuated effect was observed for kC,  
300 with a final mass loss of about 25%. ALG, XG and SC treatments controlled the  
301 fast mass loss ( $b$ ) compared to the control sample, although they had a similar  
302 mean mass loss rate at longer times, with final losses of about 25%. MC

303 resulted in a final mass loss of the same order, but with a lower mean mass loss  
304 rate after the first 2-3 day period.

305



306 **Figure 2.** Mass loss (relative to the initial cheese mass) of the coated and uncoated (control)  
307 cheese samples in terms of model components: fast loss ( $b$  parameter of the model, top  
308 segments of the bars) and slow loss after 11 days ( $a \ln(t)$ , bottom segment of the bars) for the  
309 different coating-forming systems containing, or not, (white bars) lipids (oleic acid: OA (light  
310 grey bars), or blends with beeswax (OA/BW) (dark grey bars). The experimental values after 11  
311 days were also included with the obtained LSD values.

312

313 Lipid incorporation into the coatings implied a modification in the values of the  $a$   
314 and  $b$  parameters of the fitted model, maintaining the aforementioned trends,  
315 but they did not imply, in general, a substantial reduction in the total mass loss  
316 of the samples. In fact, a multifactorial ANOVA in mass loss values (factors:  
317 polymer and lipid) and co-variable time ( $t$ ) showed a significant effect of the  
318 polymer ( $F=25.06$ ) and a less relevant one of the lipid addition ( $F=2.64$ ). The



319 statistical analysis showed three main homogenous groups: the group with the  
320 highest mass loss values included the control uncoated samples and those  
321 coated with HPMC and WPI, the second group was formed by ALG, XG and  
322 MC treatments with similar intermediate mass loss values and the third group,  
323 in which the polymers significantly reduced the mass loss, was formed by GG,  
324 kC and SC.

325 Additionally, qualitative observations which took place throughout the entire  
326 studied period showed that the SC and WPI coatings cracked during the  
327 ripening process while the ALG coatings exhibited a separation of the cheese  
328 surface, losing the required adherence. All the other coatings presented good  
329 adhesiveness and homogeneity on the cheese surface throughout storage.

330 On the basis of these analyses, homogenous coatings with the lowest mass  
331 loss values without lipids were selected to incorporate the antifungal agents.

332 These were GG and kC, although XG and MC were also selected, despite their  
333 more limited ability to control the mass loss, since antifungal agents could  
334 modify the observed tendencies.

335

### 336 **3.2 Effect of antifungals on the coating effectiveness.**

337 **Table 2** shows both the mass of adhered CFS with and without antifungal  
338 compounds after draining and the surface density of the solids. The adhesion of  
339 the CFS was generally affected by the incorporation of the antifungal  
340 compounds, which decreased in every case, except for GG, XG and MC with  
341 GT. This can be attributed to the interactions between components that modify  
342 both the viscosity of the CFS (which affects the CFS drainage) and its

343 extensibility on the cheese surface. The least affected coatings were those  
 344 formed using GG, while the kC coatings exhibited a marked reduction in the  
 345 surface density of the solids when both GT and PS were added.

346 **Table 2.** Total adhered mass of coating-forming systems (CFS, g/g cheese) and surface density  
 347 of the coating solids (g/m<sup>2</sup>) (in brackets) for the different CFS containing, or not, antifungal  
 348 agents. Variation coefficients for the adhered mass of CFS were lower than 10%.

Polymer	Without antifungal	Potassium sorbate	Gallic tannin	Aloe vera
Gellan gum	2.59 (12)	2.14 (8.5)	2.97 (12)	1.10 (4.7)
k-Carrageenan	1.94 (5.3)	0.29 (0.89)	1.28 (0.89)	-
Xanthan gum	1.28 (4.9)	1.11 (4.0)	1.39 (5.4)	-
Methylcellulose	0.40 (4.9)	0.51 (6.2)	0.51 (6.2)	-
Aloe vera	-	-	-	0.37 (1.7)

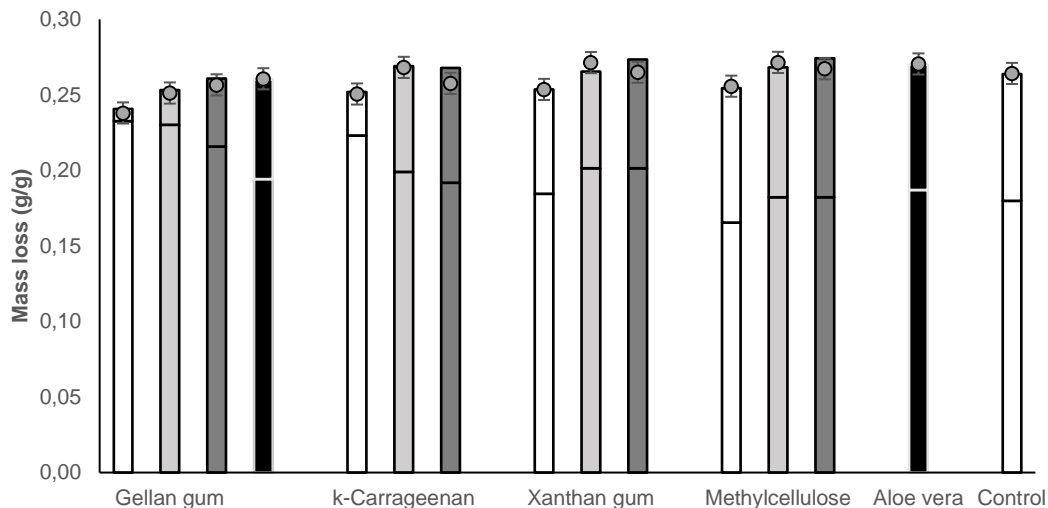
349

350 The behaviour of mass loss vs. time (**Figure 1c**) maintained the same trend as  
 351 in the initial series. However, the different amount of adhered CFS resulted in  
 352 consistent changes in the mass loss of the cheese. Thus, for treatment with kC  
 353 containing PS, the mass loss was very similar to those obtained for the  
 354 uncoated control sample as a result of the reduction in the amount of adhered  
 355 solids, which limits the barrier capacity of the coatings. The CFS of MC with GT  
 356 presented both the formation of lumps and precipitation, which make the  
 357 coating unsuitable for application.

358 Aloe vera gel that was also applied, pure or combined with GG, as cheese  
 359 coating with potential antifungal activity, exhibited a very limited capacity to  
 360 adhere to the cheese surface, resulting in low surface density of solids. When it  
 361 was applied in combination with GG, an increase in the adhered CFS and  
 362 surface density of solids was observed due to the increment of both viscosity  
 363 and solid concentration of the CFS.

364 **Eq. 2** was also fitted to the results obtained from the different treatments,  
365 demonstrating a good ability to predict the mass loss behaviour ( $r^2 > 0.99$  in all  
366 cases). **Figure 3** shows the predicted values of the sample mass loss in terms  
367 of the **Eq. 2** components (initial fast loss:  $b$  and total slow loss:  $a \ln(11)$ ), as  
368 well as the experimental mass loss values with LSD values for each treatment.  
369 PS in the GG coating promoted the fast mass loss ( $b$ ), while it reduced the  
370 subsequent mean mass loss rate ( $a$ ). GT had a similar, but more accentuated,  
371 effect in samples coated with GG, resulting in higher values of the total mass  
372 loss after 11 storage days. For samples coated with  $\kappa$ -carrageenan, both PS  
373 and GT promoted the initial fast mass loss in the samples and attenuated the  
374 subsequent mean mass loss rate. In both GG and kC treatments, the final total  
375 mass loss was higher when antifungals were present. Similar effects of  
376 antifungals were observed for XG and MC coatings.

377 The AV coating was not effective at controlling the mass loss of cheese,  
378 coherently with the low surface density of solids reached, but the blending of AV  
379 with GG improved the coating effectiveness with respect to pure AV (26.5 and  
380 27 %, respectively, as shown in Figure 3). By comparison of the mass loss  
381 values of the different coating treatments with antifungals, the GG treatments  
382 were the most effective at reducing the cheese mass loss. Therefore, the CFS  
383 based on GG were selected to study the antifungal action. Likewise, given the  
384 previously reported antifungal activity of the Aloe vera gel, these formulations  
385 were also included in the antifungal analysis.



386

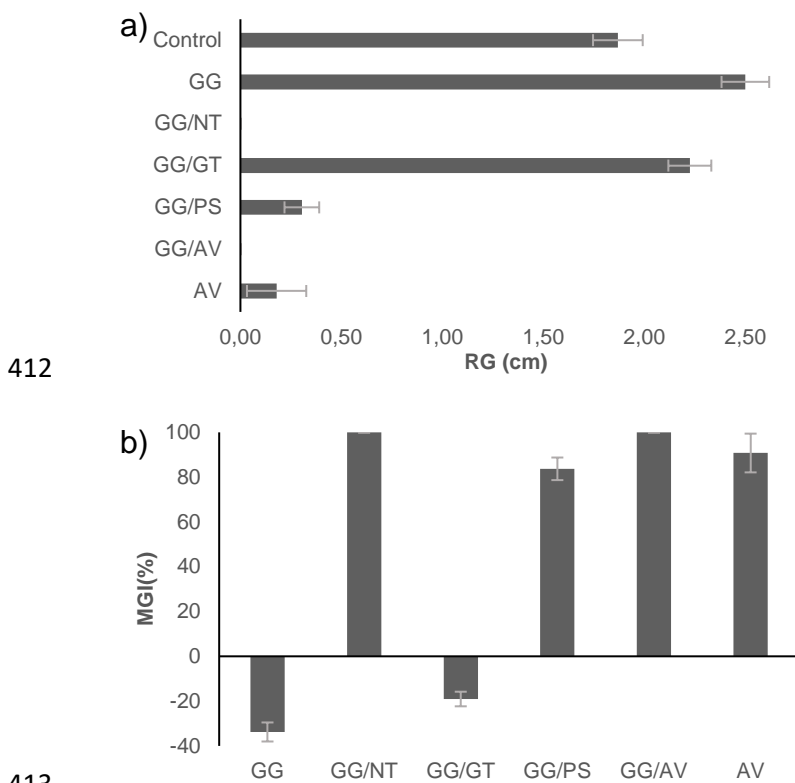
387 **Figure 3.** Mass loss (relative to the initial cheese mass) of the coated and uncoated (control:  
 388 CTR) cheese samples in terms of model components: initial fast loss (b parameter of the  
 389 model, top segments of the bars) and slow loss after 11 days (a Ln(t), bottom segment of the  
 390 bars) for the different coating-forming systems containing, or not, (white bars) antifungal  
 391 agents (potassium sorbate (light grey bars), Gallic Tannin (dark grey bars) or Aloe vera (black  
 392 bars). The experimental value after 11 days was also included with the obtained LSD values.

393

### 394 **3.3. Antifungal action of active coatings.**

395 The fungal growth radius (cm) of *Penicillium roqueforti* after 7 incubation days,  
 396 for both the uncoated samples and those coated with the selected CFS, is  
 397 shown in **Figure 4a**. The samples coated with GG without antifungals exhibited  
 398 a greater fungal growth, probably due to the surface presence of the  
 399 polysaccharide, with a great water absorption capacity and with a potential  
 400 nutritive effect for the fungus. **Figure 4b** shows the mycelial growth inhibition  
 401 (MGI) after 7 days of incubation for the selected treatments. Natamycin in GG  
 402 coatings completely inhibited the fungal growth and it eliminated the initial  
 403 colony inoculated by printing. On the other hand, the coating of GG with PS

404 showed a growth inhibition of 84%. The samples coated with pure Aloe vera  
405 showed a very high degree of inhibition whereas a complete growth inhibition  
406 was observed in the samples coated with AV/GG. The combination of GG and  
407 AV was more effective than pure AV, probably due to the higher quantity of  
408 antifungal active compound retained on the cheese surface (**Table 2**) in the  
409 combined coating associated with its higher viscosity. GG coatings with GT did  
410 not exhibit antifungal action and, as occurred with pure GG, the radial growth  
411 was greater than in the control sample.



413

414 **Figure 4.** a) Radial growth (RG) and b) mycelial growth inhibition (MGI) of *Penicillium roqueforti*  
415 after seven incubation days in inoculated cheese samples, uncoated (Control) and coated with  
416 gellan gum (GG) and with different antifungal agents (Aloe vera: AV, Potassium sorbate (PS),  
417 Gallic Tannin (GT) and Natamycin (NT)).

418

419 **4. Conclusion.**

420

421 Of the 8 biopolymers tested as to their effectiveness as coatings for cheese,  
422 gellan and xanthan gums,  $\kappa$ -carrageenan and methylcellulose were the most  
423 effective, both regarding their barrier capacity to control water loss and in terms  
424 of their integrity and adhesion on the surface of the product. Carrageenan and  
425 gellan gum had the best capacity for mass loss control, but the interactions of  
426 carrageenan and gallic tannin and potassium sorbate greatly reduced its  
427 capacity to limit water loss in the cheese. The gellan gum with both antifungals  
428 was the most effective at controlling cheese water loss during the ripening  
429 period. On the other hand, Aloe Vera gel and its mixture (1:1) with the gellan  
430 gum solution did not present a good water vapour barrier capacity, although its  
431 antifungal action against *Penicillium roqueforti* was very effective. The use of a  
432 more concentrated gel to favour the adhesion of the solids to the cheese  
433 surface should be studied in order to improve the water vapour barrier capacity  
434 of this coating. The coating of gellan with gallic tannin did not inhibit fungal  
435 growth, but with potassium sorbate it did lead to an inhibition of mycelial growth  
436 of 84%. Therefore, this last treatment could be used to prevent fungal growth  
437 during cheese ripening, while allowing control of the product mass loss.  
438 However, this is a preliminary study and the obtained results should be  
439 validated in other kinds of cheese and fungal strains.

440

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442

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