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

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

2. Materials and methods

2.1 Materials

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

2.2 Preparation of coating-forming systems (CFS)

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

The antifungal agents, potassium sorbate (PS), Gallic Tannin (GT) or natamycin (NT), were incorporated into the selected CFS in the first experimental series on the basis of the best control of the cheese mass loss during ripening. The amount of each agent to be incorporated was determined to ensure a specific final content of antifungal in the dry coating. For PS, the amount used was 0.5 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^2 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 ºC.

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.

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

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

2.4 Fungal growth inhibition analyses

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

In order to analyse the growth inhibition capacity of the selected treatments, cheese samples in 55 mm Petri dishes were covered with 2.5 g of CFS, simulating the amount of CFS adhered in the immersive application. According to the printing method described by Sapper et al.(2018), the samples were inoculated by placing a 8 mm diameter disc of a 5-day growth potato dextrose agar (PDA) culture of *Penicillium roqueforti.* Afterwards, the samples were incubated at 25 ºC 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 $$\mathrm{MGI} = \frac{DC-DO}{DC} \times 100 \qquad (1)$$

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

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

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

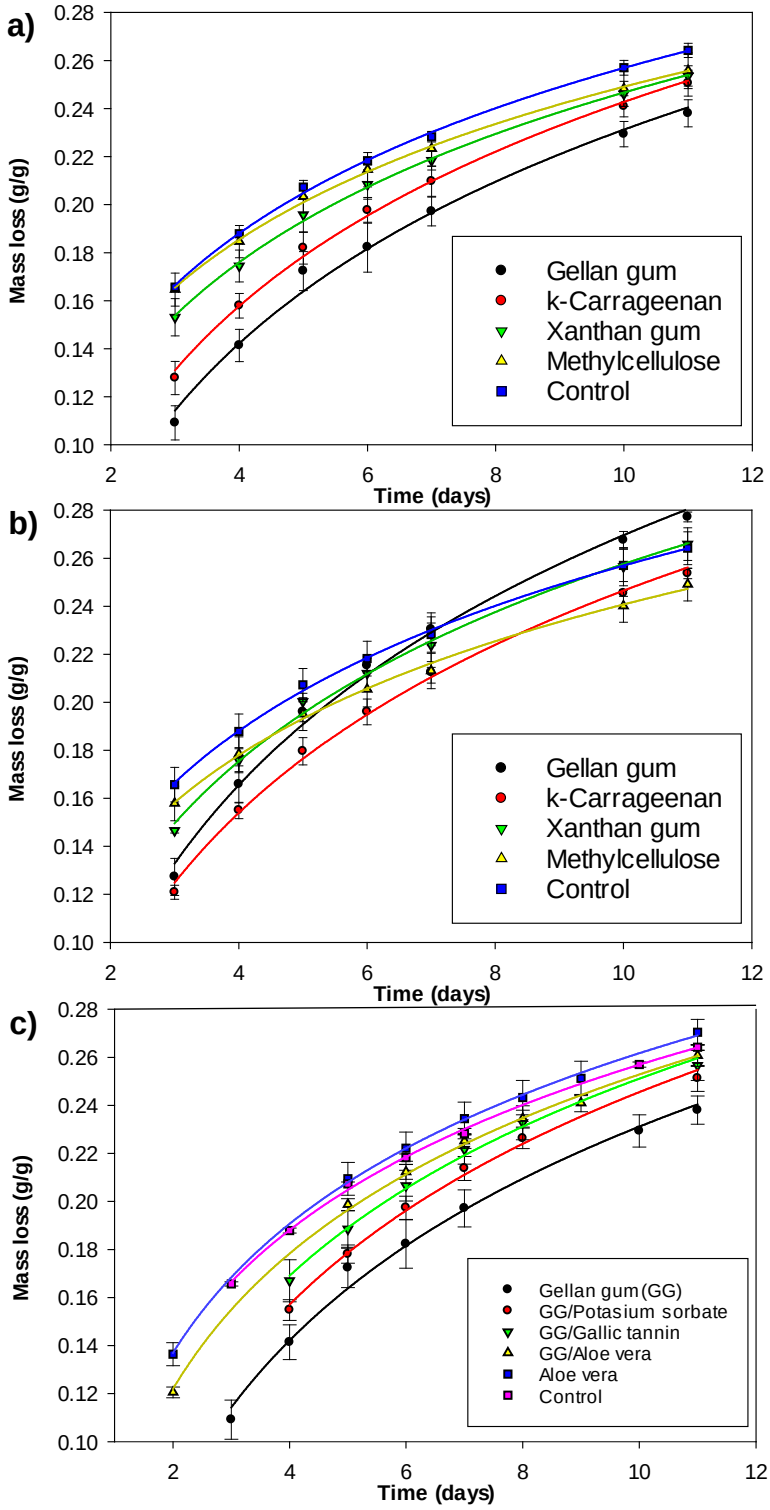
lowest values were obtained for coatings from kC, XG, HPMC and MC. The greater the surface density in the cheese surface, the higher the coating thickness and the higher water vapour barrier capacity is expected. However, potential interactions with the cheese surface could also affect the barrier capacity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

Table 1. Total adhered mass of coating-forming systems (CFS, g/g cheese) and surface density of the coating solids (g/m^2) (in brackets) for the different CFS containing, or not, lipids (oleic acid: OA, or blends with beeswax (OA/BW). Variation coefficients for the adhered mass of CFS 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)

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

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



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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.

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

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

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

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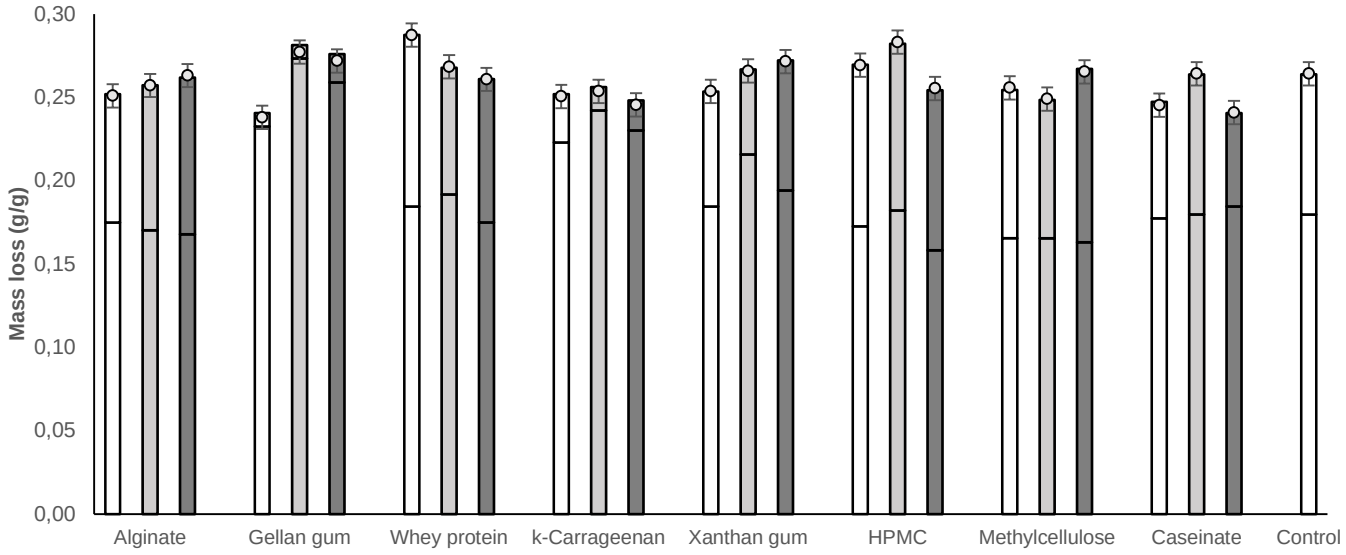

306 **Figure 2.** Mass loss (relative to the initial cheese mass) of the coated and uncoated (control)
307 cheese samples in terms of model components: fast loss (b parameter of the model, top
308 segments of the bars) and slow loss after 11 days (a Ln(t), bottom segment of the bars) for the
309 different 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

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

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

On the basis of these analyses, homogenous coatings with the lowest mass loss values without lipids were selected to incorporate the antifungal agents. These were GG and kC, although XG and MC were also selected, despite their more limited ability to control the mass loss, since antifungal agents could modify the observed tendencies.

3.2 Effect of antifungals on the coating effectiveness.

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

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

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

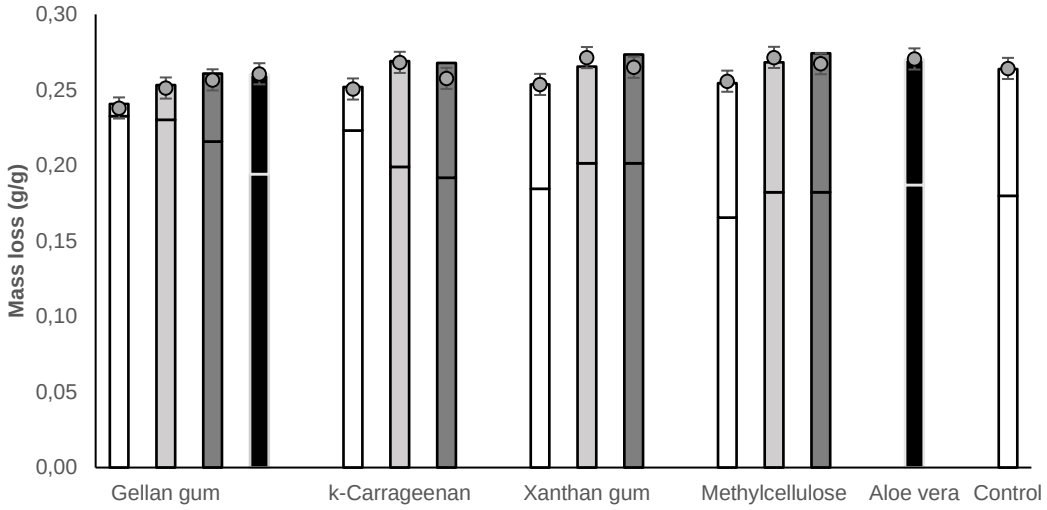

Figure 3. Mass loss (relative to the initial cheese mass) of the coated and uncoated (control: CTR) cheese samples in terms of model components: initial fast loss (b parameter of the model, top segments of the bars) and slow loss after 11 days (a Ln(t), bottom segment of the bars) for the different coating-forming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

3.3. Antifungal action of active coatings.

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

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

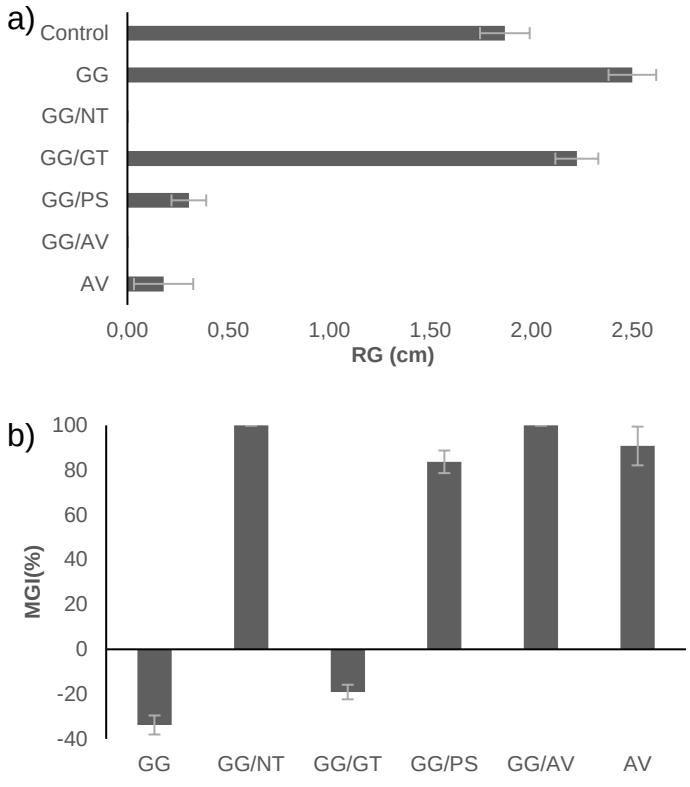

Figure 4. a) Radial growth (RG) and b) mycelial growth inhibition (MGI) of *Penicilium roqueforti* after seven incubation days in inoculated cheese samples, uncoated (Control) and coated with gellan gum (GG) and with different antifungal agents (Aloe vera: AV, Potassium sorbate (PS), Gallic Tannin (GT)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

4. Conclusion.

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

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