# Antifungal starch-based edible films containing *Aloe vera*

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**ABSTRACT**

*Aloe vera* gel, analysed as to its antifungal properties against six fungi causing plant diseases, was found to be most effective against *Fusarium oxysporum.* It was included in different ratios in starch based films plastiziced with glycerol to obtain antifungal films. These were characterized as to their physical (barrier and optical) and structural properties. Films containing the highest ratio of *Aloe vera* solids (1:1, with respect to starch) and different glycerol mass ratios with respect to starch (0.15 and 0.25) were analysed as to the water sorption and water plasticization effects, in order to discover the water sensitivity of the films in different RH ambients. Films with 0.15 g glycerol/g starch were more homogenous, with a lower degree of starch crystallization, reduced water vapor permeability and higher gloss and transparency. These films with the highest *Aloe vera* ratio were effective at controlling fungal decay and weight loss in cherry tomatoes.

**Keywords:** *in vitro* assays, *in vivo* assays, weight loss, physical properties, structural properties

## 1. INTRODUCTION

Protecting fruits and vegetables from fungal infection and growth is a crucial concern for the purposes of promoting the extension of their shelf life and ensuring product quality and safety (Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999). Fungal attack causes heavy losses in plant products while also compromising the health of consumers because of the production of micotoxins. Fruit decay caused by fungal spoilage is the main postharvest problem, leading to serious economic losses, especially in the case of more sensitive plant products. Treatments with synthetic fungicides are being restricted, due to their potential toxicological effects on consumers, and non-toxic, natural treatments need to be developed. In this sense, some plant extracts exhibit antifungal properties and their use in the preservation of fruits and vegetables could be an interesting alternative (Valverde, Valero, Martínez-Romero, Guillén, Castillo, *et al*., 2005; Martínez-Romero, Alburquerque, Valverde, Guillén, Castillo, *et al*., 2006; Misir, Brishti, & Hoque, 2014). *Aloe vera* gel (the colourless mucilaginous fraction obtained from the parenchymatous cells of the fresh leaves of Aloe spp.) is a complex matrix with bioactive properties (Choi & Chung, 2003; Ezuruike & Prieto, 2014). It is rich in anthraquinones (aloe-emodin, aloetic acid, aloin, anthranol, barbaloin, isobarbaloin, emodin and ester of cinnamic acid), saccharides (cellulose, glucose, mannose, aldopentose, acetylated mannan, glucomannan, acetylated glucomannan, galactogalacturan, glucogalactomannan, galactoglucoarabino-mannan), vitamins (B1, B2, B6, C, β-carotene, choline, folic acid and α-tocopherol), enzymes (amylase, carboxypeptidase, catalase, cyclooxydase, lipase and oxidase) and other low-molecular-weight substances (Choi & Chung, 2003). Several components, such as glycoprotein, barbaloin, emodin, mannose-6-phosphate, acemannan, aloesin, β-sitosterol or diethylhexylphthalate, have pharmacological activity. Likewise, their antimicrobial activity against several bacteria has been demonstrated. *Aloe vera* gel was effective against *Streptococcus pyogenes* and *Streptococcus faecalis* (Heggers, Pineless, & Robson, 1979; Robson, Heggers, & Hagstrom, 1982) and the *Aloe vera* exudate had positive antibacterial effects against *S. agalactiae*, *Citrobacter sp*., *Serratia marcescens*, *Enterobacter aerogenes*, *Enterobacter sp*., *Bacillus subtilis*, *Staphylococcus aureus H*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Corynebacterium xerose*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (George & Pandalai, 1949; Gottshall, Lucas, Lickfeldt, & Roberts, 1949; Lorenzetti, Salisbury, Beal, & Baldwin, 1964; Bruce, 1967; Heggers, *et al*., 1979; Heck, Head, Nowak, Helm, & Baxter 1981; Levin, Hazenfratz, Friedman, Palevitch, & Perl, 1988;

Stuart, Lefkowitz, Lincoln, Howard, Gelderman, *et al*., 1997). Ethanol extract inhibited the growth of *Klebsiella pneumoniae* and *Klebsiella sp*. (Reynolds & Dweck 1999). Previous studies also showed that *Aloe vera* was effective against different plant pathogenic fungi. It reduced the spore survival of *Penicillium*, *Botrytis* and *Alternaria* by 15–20% (Saks and Barkai-Golan, 1995), and inhibited the mycelium growth of *Rhizoctonia*, *Fusarium* and *Colleotrichum* by 22–38% (Jasso de Rodríguez, Hernández-Castillo, Rodríguez-García, & Angulo-Sánchez, 2005). Castillo, Navarro, Zapata, Guillén, Valero, *et al*. (2010) also reported an inhibition effect of *Aloe vera* gel on the mycelium growth of *Penicillium digitatum* and *Botrytis cinerea*. The availability, non-toxic nature and potential bioactivity of the *Aloe vera* products make them an interesting alternative for the purposes of controlling fungal diseases in fruits and vegetables under both pre and postharvest conditions.

The application of active compounds included in biopolymer matrices (active coatings) has different advantages, such as the dosage adjustment, cost reduction and greater product adherence on the plant surface. Therefore, the coating forming ability of biopolymer matrices contributes to an enhancement in product quality, since coating reduces the respiration and transpiration rates of the plant products, thus retarding their senescence and extending their shelf life. Antifungal biopolymer coatings would protect fruits and vegetables from the rapid deterioration associated with the weight loss, colour changes and softening that occur under post-harvest conditions; these are accompanied by the occurrence of fungal decay mainly due to species of different fungi of the genera *Penicillium*, *Botrytis*, *Monilia*, among others (Martínez-Romero *et al*., 2006; Castillo, *et al*., 2010).

Polysaccharides and proteins, obtained from biomass, can be used for the formulation of edible films for coating purposes in fruits and vegetables (Lee, Shim, & Lee, 2004; Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). In particular, starch-based materials have been widely studied since they are edible, food compatible and could offer inexpensive solutions (Bastioli 2001; Ortega-Toro, Morey, Talens, & Chiralt, 2015). Starch is obtained from renewable resources, widely available and low cost and it can be used to obtain edible films for food applications, with low oxygen permeability (Ortega-Toro, Morey, Talens, & Chiralt, 2015).

Therefore, the formulation of starch-based coatings containing *Aloe vera* as antifungal component is an appealing alternative for the purposes of extending the shelf life of fruits and vegetables. In this sense, the aim

of the present study is to obtain antifungal starch based films for fruit coating applications by using *Aloe vera* gel. The antifungal capacity of *Aloe vera* gel against six plant-disease-causing fungi was analysed and, afterwards, starch-based coating films with *Aloe vera* were characterized as to their physical and structural properties and their preservation capacity and antifungal activity in cherry tomatoes.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Fresh *Aloe vera* gel (*Aloe barbadensis Miller*) was obtained under aseptic conditions using the mucilaginous part of the leaf and immediately cold stored. Pregelatinised corn starch was supplied by Roquette (Roquette Laisa, Benifaió, Spain). Other analytical grade reagents were provided by Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

### 2.2 Antifungal properties of *Aloe vera* gel

The antifungal properties of *Aloe vera* gel were analysed before formulating starch-based materials. The fungi tested were *Fusarium oxysporum* CECT 2715, *Alternaria alternate* CECT 20923, *Colletrotrichum gloesporoides* CECT 20250, *Bipolaris spicifera* CECT 2776, *Curvularia hawaiiensis* CECT 20934 and *Botryotinia fuckeliana* CECT 2100. The fungi were kept on potato dextrose agar (PDA) and transferred periodically to maintain active growth.

The bioassay was performed in Petri dishes (90mm x 15mm or 150mm x 20mm). Fresh *Aloe vera* gel, with a moisture content of 94.3 ± 0.5%, was mixed (in 1:1 wt. ratio) and homogenized with previously sterilized Potato Dextrose Agar (PDA) growth medium at 45-50ºC, while the medium was still liquid, and distributed in the Petri dishes. Then, these were inoculated with an 8 mm diameter disk of 5-day old colony on PDA of each fungus. Plates were incubated in the dark at 25ºC for 7 days. The Petri dish control contained only PDA medium. Fungal growth was evaluated by daily measuring the diameter of the colonies in two perpendicular directions and the growth rate was estimated. Six replicates per treatment and control samples were carried out. Mycelial growth inhibition (MGI) was calculated at 7 days of incubation, using equation 1 (Albuquerque, Camara, Mariano, Willadino, Júnior, *et al*., 2006).

$$MGI = \frac{DC - DO}{DC} x100 \quad (1)$$

Where, DC is the average diameter of the colonies in the control plates and DO is the average diameter of colonies in the plates containing the active component.

**2.3 Film formulation containing *Aloe vera***

Starch-based films containing different ratios of *Aloe vera* were used in order to analyse the effect of the active on the film's functional properties as coating material. Pregelatinized corn starch was water dispersed with glycerol, as plasticizer, in different ratios (0.15 and 0.25 with respect to starch) as well as with *Aloe vera* gel, using the following ratios of dry solids of *Aloe vera* :starch: 1:3, 1:2 and 1:1, thus obtaining 6 different formulations (F1 to F6). Mass fractions of the dry film components for all formulations are shown in Table 1. Dispersions were homogenised using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany) for 2 min at 13500 rpm plus 3 min at 20500 rpm. Films were obtained by casting. The mass of the formulations containing 1.5 g of total solids was spread evenly over a Teflon casting plate of 15 cm diameter resting on a level surface. Films were formed by drying for approximately 72 h at 45% RH and 20 °C. Dry films could be peeled intact from the casting surface and they were conditioned for 1 week at 75.7% RH and 10 °C for the purposes of determining their physical and structural properties. The RH in desiccators was controlled by using an oversaturated solution of sodium chloride (Panreac Quimica, S.A.).

**2.4 Functional properties of the films.**

The water vapour barrier capacity, gloss and transparency of the films were determined as relevant characteristics of the films as fruit coatings. All measurements were taken in triplicate for each sample and three films were measured from each formulation. Film thickness was measured with a Palmer digital micrometre (Palmer-Comecta, Spain, ± 0.001 mm) at six random positions in the film. The moisture content of films conditioned at 75.7% RH and 10 °C (near the fruit cold storage conditions) was determined from the mass loss after drying them in a natural convection oven (J.P. Selecta, S.A., Barcelona, Spain) at 60 °C for 24 h and subsequent conditioning in a $P_2O_5$ desiccator for 8 days.

Water vapour permeability (WVP) was measured in films conditioned for 1 week in hermetic desiccators at 10 °C and 75.7% RH, using a 75.7-100 % RH gradient and 10ºC, by using the ASTM E96-95 (ASTM, 1995)

gravimetric method, taking into account the modification proposed by McHugh, Avena-Bustillos, & Krochta (1993). Distilled water was placed in Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium) to expose the film to 100% RH on one side. Once the films were secured, each cup was placed in a RH (75.7%) equilibrated cabinet at 10 °C, with a fan placed on the top of the cup to reduce resistance to water vapour transport, thus avoiding the stagnant layer effect in this exposed side of the film. The RH of the cabinets (75.7%) was held constant using oversaturated solutions of sodium chloride. The free film surface during film formation was exposed to the lowest RH. The cups were weighed periodically (0.0001 g) and water vapour transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film area. From the WVTR data, the vapour pressure on the film's inner surface ($p_2$) was obtained with Eq. (2), proposed by McHugh *et al*. (1993), to correct the effect of concentration gradients established in the stagnant air gap inside the cup. The water vapour permeance was calculated using Eq. (3) as a function of $p_2$ and $p_3$ (pressure on the film's outer surface in the cabinet). The permeability was obtained by multiplying the permeance by the average film thickness.

$$WVTR = \frac{PDLn\left[\left[\frac{P-p_2}{P-p_1}\right]\right]}{RT\Delta z} \quad (2)$$

where $P$, total pressure (atm); $D$, diffusivity of water through air at 10 °C ($m^2/s$); R, gas law constant (82.057 x $10^{-3}$ $m^3$ atm $kmol^{-1}$ $K^{-1}$); $T$, absolute temperature (K); $\Delta z$, mean stagnant air gap height (m), considering the initial and final z values; $p_1$, water vapour pressure on the solution surface (atm); and $p_2$, corrected water vapour pressure on the film's inner surface (atm).

$$permeance = \frac{WVTR}{p_2 - p_3} \quad (3)$$

The optical properties of the films were also determined. The gloss was measured on the free film surface, at an incidence angle of 60° by means of a flat surface gloss meter (Multi Gloss 268, Minolta, Germany),

following the ASTM standard D523 method (ASTM 1999). The results were expressed as gloss units (GU), relative to a highly polished surface of black glass standard with a value near to 100 GU.

The transmittance of the films was determined over the whole UV-VIS range in film samples (1cm x 3 cm) equilibrated at 25 °C and 75.7% RH, using an UV-VIS spectrophotometer (Perkin Elmer Instruments, Lambda 35, Waltham, USA), within a wavelength range of between 200 and 1000 nm.

**2.5 Structural properties of films**

The microstructural analysis of the cross-sections and surface of the films was carried out using a scanning electron microscope (JEOL JSM-5410, Japan). The film samples were maintained in desiccators with $P_2O_5$ for 2 weeks to guarantee that water was not present in the sample. Film pieces, 0.5 $cm^2$ in size, were cryofractured from films and fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

A diffractometer (XRD, Bruker AXS/D8 Advance) was used to obtain the X-ray diffraction patterns and degree of crystallinity. All the samples (equilibrated for one week at 10 °C and at 75.7% RH) were analysed, between 2θ: 5° and 2θ: 30° using KCu radiation (λ: 1.542 Å), 40 kV and 40 mA with a step size of 0.05°. For this analysis, samples were cut into 4 cm squares. The degree of crystallinity of starch matrices was calculated as the ratio between the absorption peaks and the total diffractogram area, expressed as a percentage, by using OriginPro 8.5 software. Moreover, basal spacing for starch crystallites was calculated by applying Bragg's Law (Eq. (4)).

$$n\lambda: 2dsin\theta \quad (4)$$

Where, *n*, is a whole number; $\lambda$ is the wavelength of the rays; $\theta$ is the angle between the incident ray and the surface of the crystal and *d* is the spacing between layers of crystals

**2.6 Water sorption and water plasticization of the films**

Formulations with the greatest ratio of *Aloe vera* gel were selected for the purposes of studying their water sorption capacity and plasticization effect, since the material is water sensitive and the equilibrium relative

219 humidity might affect its functionality, depending on whether it is in a glassy or rubbery state according to the
220 different storage conditions. To this end, films were equilibrated at different relative humidities (RH) or water
221 activities ($a_w$) to determine both the water sorption isotherms and the glass transtion temperature (Tg). Three
222 film samples of each formulation (1.5 - 2.0 g), accurately weighed, were placed in desiccators at 10 °C (near
223 the fruit cold storage conditions) and equilibrated at different $a_w$ using oversaturated solutions of LiCl, $MgCl_2$,
224 $K_2CO_3$, $Mg(NO_3)_2$, $CuCl_2$ and NaCl (different $a_w$ values are shown in Figure 5). Samples were weighed
225 periodically (0.00001 g precision) for 3 weeks, when the equilibrium was reached. Finally, the equilibrium
226 moisture content of each sample was determined by drying them in a vacuum oven at 60 °C for 24 h and
227 subsequently conditioning them in a $P_2O_5$ desiccator for 8 days. The experimental sorption isotherms were
228 fitted to the Guggenheim-Anderson-de Böer (GAB) model (Eq. (5)) over the entire $a_w$ range.
229

$$we = \frac{w_0.C.k.a_w}{(1-K.a_w).(1+(C-1).k.a_w)} \quad (5)$$

230

231 In Eq. (5), *we* is the equilibrium moisture content on dry basis, $a_w$ is water activity, $W_0$ is the monolayer
232 moisture contentand *C* and *k* are equation parameters, both of which are temperature dependent and related to
233 the water sorption energy.
234 The glass transition temperatures of the films equilibrated at the different RH were determined by Differential
235 Scanning Calorimetry (DSC). These analyses were carried out using a DSC (DSC 1 Stare System, Mettler-
236 Toledo, Inc., Switzerland). Samples (~20 mg) were placed into aluminium pans and hermetically sealed. All
237 of the pans were then heated by double scan between -60 °C and 120 °C in order to analyse phase transitions.
238 The temperature range for each sample was fitted according to its $a_w$. In general, samples were cooled from
239 room temperature to a temperature at least -60 °C below its Tg and then, a heating scan was performed. Both
240 cooling and heating scans were carried out at 10 °C/min. An empty aluminium pan was used as reference. The
241 Tg was determined as the midpoint temperature of the glass transition. Each sample was analysed in triplicate.
242 The Gordon and Taylor model (Gordon & Taylor, 1952) was used for the purposes of modelling Tg values as
243 a function of the sample moisture content (Eq. (6)).
244

$$Tg = \frac{(1 - X_w).Tg_s + k.X_w.Tg_w}{X_s + kX_w} \quad (6)$$

where *Tgs*, *Tgw*, *Tg* are glass transition temperatures of solids, water and their mixture, respectively, *Xw* is the mass fraction of water, and *k* is the Gordon-Taylor parameter, from which the thermodynamic standpoint is equivalent to the ratio of the change of component mixture specific heat at their Tg (Mrad, Bonazzi, Courtois, Kechaou, & Mihoubi, 2013).

**2.7 *In vivo* test of coating formulations**

How well the formulation with the greatest ratio of *Aloe vera* and the lowest ratio of glycerol, potentially more active and less plastiziced, controlled fungal decay and preserved quality was tested on cherry tomatoes and compared with the non-coating application. Tomatoes were selected according to their degree of ripeness and absence of defects. They were washed with 1% sodium hypochlorite for 2 min and subsequently washed twice in sterilised water. The fungus (*Fusarium oxysporum*) was inoculated both before (curative treatment) and after (preventive treatment) the fruit coating with the film forming dispersion. The inoculation was carried out by using a sterilised needle to transfer 5 μL of a $10^6$ espores/ml suspension. The coating-forming dispersion was sprayed on the fruit surface and dried under room conditions. When the coating was dry, the tomato samples were stored in cabinets at 10 °C and 85% RH (using an oversatured solution of KCl) for 7 days. Afterwards, the samples were stored at 25 °C and 85% RH for another 7 days to simulate cold storage conditions and subsequent room temperature after consumption. A visual inspection was carried out every 5 days to determine fruit appearance and fungal incidence during storage. In parallel, weight loss in the non-inoculated fruits (coated and uncoated) was also controlled. Twenty tomatoes were considered in each series (coated and non-coated fruits).

**2.8 Statistical analysis**

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used to carry out statistical analyses of data through analysis of variance (ANOVA). Fisher's least significant difference (LSD) was used at the 95% confidence level.

## 3. RESULTS

### 3.1 Antifungal properties of *Aloe vera* gel

Figure 1 shows the radial growth of different fungi incubated on PDA with and without *Aloe vera* gel. The Mycelium Growth Inhibition (MGI) values on the 7th day of incubation at 25°C and the linear equation fitted to the growth data were also shown in each plot. The slope of the straight line describes the growth rate of each fungus in the different media with and without *Aloe vera* gel. In almost every case, the growth rate of the fungi decreased when *Aloe vera* was present in the medium, which indicates its inhibition effect on the growth of fungi. A high degree of inhibition was observed in the cases of *Fusarium oxysporum* (MGI: 65.16), *Bipolaris spicifera* (MGI: 53.09) and *Curvularia hawaiiensis* (MGI: 43.21). In contrast, *Colletrotrichum gloesporoides* showed the lowest MGI values, while no notable decrease in the growth rate with respect to the control was observed. However, the greatest inhibition occurred for *Fusarium oxysporum*, where a growth rate of 1.6 $mm \cdot day^{-1}$ was observed against 6.1 $mm \cdot day^{-1}$ in the control plate. Similar results of *Aloe vera* pulp on *Fusarium oxysporum* were reported by De Rodríguez, Hernández-Castillo, Rodríguez-García, & Angulo-Sánchez (2005) when studying the *in vitro* antifungal activity of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi (*Rhizoctonia solani*, *Fusarium oxysporum*, and *Colletotrichum coccodes*). These results point to the antifungal activity of *Aloe vera* gel and its potential as an active component in fruit-coating formulations. *Fusarium oxysporum* is a large genus of filamentous fungi widely distributed in soil and plants which provokes several diseases as well as the production of mycotoxin in cereals, fruits and vegetables. Of these, tomatoes plants and fruits are especially affected by *Fusarium oxysporum* (Lagopodi, Ram, Lamers, Punt, Van den Hondel, *et al*., 2002; Houterman, Speijer, Dekker, De Koster, Cornelissen, *et al*., 2007) and both pre- and post-harvest treatments using coating-forming products containing *Aloe vera* gel could benefit their production and commercialization processes.

### 3.2 Functional properties of starch-based films

In order to produce starch-based antifungal films/coatings with *Aloe vera*, the ratio of active component must be fitted in order to obtain the best antifungal action with adequate film functional properties. In this sense, what would be desirable is the maximum ratio of active component that allows for film formation with good barrier and mechanical performace. To analyse the effect of the *Aloe vera* and plasticizer content on film

functionality, the six film formulations shown in Table 1 were obtained and characterised. Table 1 also shows the film properties in terms of thickness, equilibrium water content and water vapour permeability (WVP), gloss and transparency when at 75.7 % RH and 10 °C (conditions near to what is required for the post-harvest preservation of fruit). All of the film formulations were of similar thickness (about 65 μm), whereas the equilibrium moisture content slightly decreased when the *Aloe vera* ratio rose or the glycerol content decreased, F6 exhibiting the lowest water adsorption capacity. Coherently, the WVP also decreased in line with this compositional change, the least permeable being films with the highest ratio of *Aloe vera* and the lowest glycerol content. However, the differences were not relevant from a practical point of view.

In general, films exhibited low gloss values for every film formulation, but this property slightly increased at the highest ratio of *Aloe vera* with the lowest glycerol content. As concerns transparency in the UV-VIS range, all of the films were opaque up to 290 nm, from which transmittance reached a plateau without any specific absorbance peaks. Table 1 shows the transmittance value at 450 nm as an indicator of the different film transparency. A higher glycerol ratio gave rise to lower transmittance values, which suggests a more heterogenous microstructure promoting light dispersion. Likewise, the highest ratio of *Aloe vera* enhanced film transparency probably due to its effect on the film's microstructure (starch crystallization and polymer chain arrangement) which determined the light dispersion-transmission ratio. The differently entangled, or crystalline structures affect the morphology and anisotropy of the material giving rise to distinct light interactions and transparency. In the next section, the microstructural features in the films are analysed. Previous studies into the effect of different glycerol contents in starch-based materials reported phase separation when the amount of glycerol exceeded a critical value (Forssell, Mikkilä, Moates, & Parker 1997; Lourdin, Bizot, & Colonna, 1997). This aspect could affect the film microstructure and optical properties.

### 3.3 Structural properties of studied films

Figure 2 shows cross-section micrographs of the studied films conditioned at 75.7% RH and 10 °C. Different amounts of glycerol induced marked changes in the morphology of the cryofracture surface of the films. Globular formations appeared in films with the highest ratio of glycerol, whereas a smoother fractured surface can be observed in the other films. These formations were bigger when the *Aloe vera* content rose, which points to the phase separation of starch polymers, *Aloe vera* constituents and glycerol, as previously reported

for starch based materials with high glycerol content which promoted phase separation (Forssell, Mikkilä, Moates, & Parker 1997; Lourdin, Bizot, & Colonna, 1997). This is coherent with the fact that the films with the highest ratio of the plastizicer had the lowest gloss and transparency values. Glycerol reduces the solvent capacity of water for the polymers present, which could provoke the exclusion effect and polymer phase separation, even in the water solution. This separation affected the microstructure of the films, giving rise to different domains of the macromolecular components present. When the glycerol ratio decreased, a better polymer compatibility was obtained and films showed a more homogenous network, which, in turn, exhibited more isotropic properties. Greater material isotropy enhanced light transmission rather than dispersion, increasing transparency rather than opacity. The glycerol and *Aloe vera* contents could also affect the degree of crystallinity of amylose in the dried films, which could also contribute to the film's optical behaviour.

Figure 3 shows the X-ray diffraction pattern of the different film formulations conditioned at 75.7% and 10 °C. The degree of crystallinity (Xc) of the formulations was calculated as the ratio between both the area under crystalline peak and the total area under the difractograms, which include the amorphous response. *Aloe vera* powder exhibited a completely amorphous pattern, whereas *Aloe vera*-free starch films with a 0.25:1 glycerol- starch ratio (control film) exhibited a similar crystallization pattern and Xc values to that previously reported for corn starch films obtained either by casting (Ortega-Toro, Jiménez, Talens, & Chiralt, 2014) or thermoprocessed (Ortega-Toro, Santagata, Gomez d'Ayala, Cerruti, Talens, *et al.*, 2016; Ortega-Toro, Collazo-Bibliardi, Talens, & Chiralt, 2016). The films obtained by casting exhibit a higher degree of crystallinity due to the length of time required for the film drying when amylose mainly crystallized in the high molecular mobility context offered by the solvent. The main diffraction peaks appeared at 2θ: 12, 17, 20 and 22°, corresponding to an interplanar distance of 7.4, 5.2, 4.4 and 4.0 nm.

Incorporating *Aloe vera* and increasing the glycerol content did not affect the diffraction peaks but did modify the degree of crystallinity in the films. Gycerol plasticizing promoted amylose crystallization during the film drying step due to the enhancement of molecular mobility, thus favouring the aggregation of amylose helical forms (Rindlav-Westling, Stading & Gatenholm, 2002). Therefore, the films with the highest glycerol content exhibited a more crystalline structure. In contrast, the incorporation of *Aloe vera* limited amylose crystallization due to the hindering effect provoked by polymer blending, mainly when the polymer compatibility was enhanced at the lowest glycerol ratio. Moreover, a lower content of glycerol is desirable

because the greater molecular mobility could also provoke unwanted changes in the functional properties of films throughout the storage time.

### 3.4 Water and glycerol plasticization

According to the obtained functional properties of starch-based films and taking the antifungal properties of *Aloe vera* gel into account, the formulations with the highest content of the gel (F5 and F6) would be more adequate as active coatings on fruits and vegetables. Then, the effect of water gain or loss of the coatings must be analysed in order to discover how film equilibration under different RH conditions after coating applications could affect their functional properties, depending on their glassy or rubbery state. Glassy, brittle coatings are not desirable since they break easily losing their protective function. Likewise, excessively plasticized coatings are less efficient water vapor barriers. Figure 4 shows the sorption isotherms of starch-based films containing two ratios of glycerol and *Aloe vera* at 10°C. The experimental points and the Guggenheim-Anderson-de Boer (GAB) fitted model are shown. Likewise, the values of glass transition temperature (Tg) at each $a_w$ value are indicated, as well as the fitted linear relationship. As reported by other authors (Mali, Sakanaka, Yamashita, & Grossmann, 2005), a higher glycerol content promoted the water sorption capacity of the films, as deduced from the higher values of the equilibrium moisture at the different $a_w$ values. Likewise, the plastizicing effect of glycerol in the matrix can be observed in the lower Tg values at each $a_w$ of the films with 0.25 g glycerol/g starch. Figure 5 shows the Tg values of the two films as a function of their water content as well as the parameters ($K$ and $Tg_s$) obtained from the fitting of the Gordon and Taylor model to the experimental values. As expected, the Tg of anhydrous solids ($Tg_s$) decreased when the glycerol content rose in the film, whereas the water plasticization effect was more marked (higher k value) in the films with the lowest glycerol ratio, which makes them slightly more water sensitive. This behaviour agrees with that previouisly reported by Chaudhary, Adhikari, & Kasapis (2011) for glycerol or xylitol plasticized starch films. There are a threshold water content within the starch matrix, beyond which there is competitive interaction between water and plasticizer for starch interaction and excess water within starch matrix does not significantly reduce Tg by plasticizers.

As can be observed in Figure 4, F5 and F6 films at 10ºC will be in a rubbery state if the RH of equilibrium is above 53 and 66 %, respectively, but become glassy in drier conditions. By applying the GAB and Gordon &

383 Taylor models, the critical moisture contents and the critical water activities ($a_{wc}$) were estimated for each
384 film, according to previous studies for different kind of products (Moraga, Martínez-Navarrete & Chiralt,
385 2006; Fabra, Talens & Chiralt, 2010; Mrad *et al*., 2013). The $a_{wc}$ values were 0.53 (F5) and 0.66 (F6) while
386 the critical moisture contents ($We_c$) at 10ºC were 0.14 (F5) and 0.17 (F6) g /g dry film.
387 High RH values are usually employed to preserve fruits and vegetables during post-harvest storage; in field
388 applications, however, films could suffer fractures at low ambient RH due to their glassy state. Under
389 conditions slightly above the $a_{wc}$ or $We_c$, coating materials will be neither brittle nor so sticky. F5 and F6
390 films conditioned at a RH of over 70% will be rubbery, and therefore flexible. Thus, these materials could be
391 used as coatings for fruits and vegetables stored under refrigeration conditions (10°C and ~85% RH).
392 Nevertheless, considering the better performance of the F6 formulation in terms of water vapour barrier and
393 optical parameters, this was the one selected to analyse the preservation capacity and antifungal power when
394 applied on cherry tomotoes. This formulation also showed a $a_{wc}$ value that was nearer the usual RH found
395 during the storage of fruits and vegetables and less stickiness would be expected in the films under these
396 conditions.
397
398 **3.5 Coating application on cherry tomatoes.**
399 The F6 formulation with the lowest amount of glycerol (the most appropriate for high RH storage) and the
400 highest ratio of *Aloe vera* gel (better antifungal effectiveness) was selected for the purposes of analysing its
401 capacity to preserve cherry tomatoes.
402 Figure 6 shows the weight loss of uncoated and coated cherry tomatoes stored at 10°C and 85% RH for 7
403 days, and subsequently stored at 25 °C and 85% RH for another 7 days. The storage temperature was changed
404 in order to simulate conditions before and after the commercialization of these fruits. A linear weight loss
405 tendency was observed in both periods with different slopes (rate). Coating greatly retards weight loss in the
406 fruits throughout both periods, and especially at 25ºC. After the 14th day of storage, the tomatoes without
407 coatings exhibited a weight loss that was 84 times greater than the coated tomatoes. This result is highly
408 positive for the post-harvest preservation of the fruits. In fact, coatings containing *Aloe vera* gel were reported
409 to increase the shelf-life of different fruits, such as *Carica papaya* (Marpudi, Abirami, Pushkala & Srividya,

2011), grapes (Valverde *et al*., 2005), *Ananas Comosus* (Adetunji, Fawole, Arowora, Nwaubani, Ajayi, *et al*., 2012) or tomatoes (Chauhan, Nanjappa, Ashok, Ravi, Roopa, *et al*., 2015).

The parallel experimental series, the aim of which was to analyse the antigungal effect of the F6 coating in fruits inoculated with *Fusarium oxysporum* and stored under the same conditions for 14 days, also revealed a positive effect of the coating on fruit safety and quality. Table 2 shows the data obtained for the fruit's appearance, its softness and fungal incidence after the different treatment, as deduced form the visual inspections carried out every five days. On the $5^{th}$ day of storage, all of the tomatoes with preventive and curative coatings exhibited a good appearance, whereas 20% of the uncoated tomatoes had soft zones. There were no cases of fungal decay at this time. On the $10^{th}$ day of storage, all of the treatments were affected by *Fusarium oxysporum* and exhibited soft zones, but the control samples were the ones that were most affected by the presence of fungus (50 % of the fruits). At the end of storage ($14^{th}$ day), all of the cherry tomatoes in the control treatment were affected by softening or fungus infection; however, of the fruits with preventive and curative coatings, 30% and 40% respectively, exhibited a good appearance. Therefore, these coatings improved the preservation of cherry tomatoes by reducing fungal incidence and represent a promising solution for the purposes of preserving similar fruits.

## 4. CONCLUSIONS

*Aloe vera* gel was effective at controlling the growth of several fungi, exhibiting the greatest efficacy against *Fusarium oxysporum*. It could be effectively incorporated into starch matrices to obtain antifungal coatings at a ratio of *Aloe vera* solids-starch up to 1:1, using glycerol as plasticizer. Films with 0.15 g glycerol/ g starch were more homogenous than those containing 0.25 g/g starch, exhibiting lower starch crystallization, reduced water vapor permeability and higher gloss and transparency. The films with the highest ratio of *Aloe vera* and the lowest amount of glycerol have a critical water activity of 66 % at 10ºC, which implies that under the ususal RH conditions for fruit and vegetable post-harvest preservation, the coatings will be flexible, thus preventing cracks. These coatings were effective at controlling fungal decay and weight loss in cherry tomatoes, and so can be a natural, non-toxic alternative to synthetic fungicides in the preservation of fruits and vegetables

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557

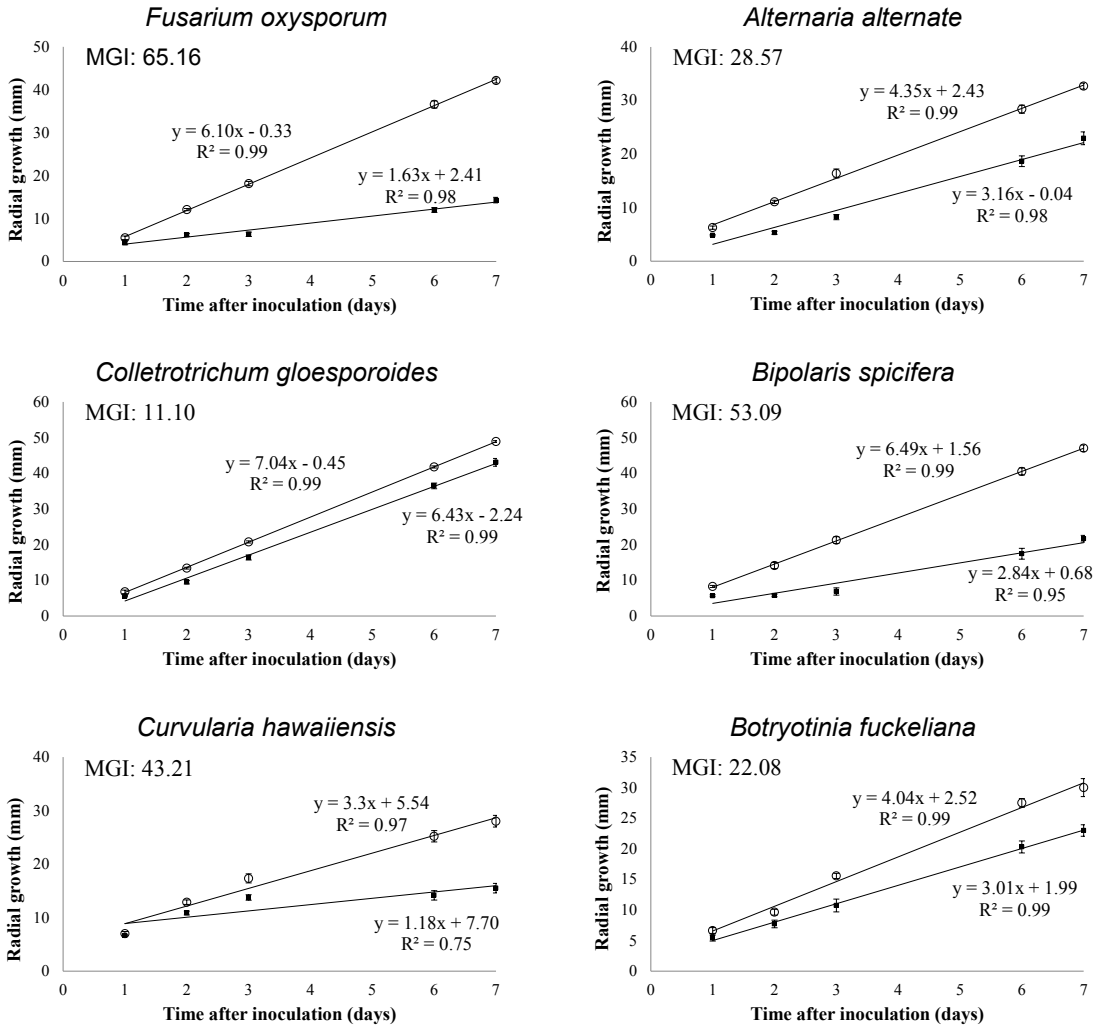
Fusarium oxysporum
MGI: 65.16
y = 6.10x - 0.33
R² = 0.99
y = 1.63x + 2.41
R² = 0.98
Alternaria alternate
MGI: 28.57
y = 4.35x + 2.43
R² = 0.99
y = 3.16x - 0.04
R² = 0.98
Colletrotrichum gloesporoides
MGI: 11.10
y = 7.04x - 0.45
R² = 0.99
y = 6.43x - 2.24
R² = 0.99
Bipolaris spicifera
MGI: 53.09
y = 6.49x + 1.56
R² = 0.99
y = 2.84x + 0.68
R² = 0.95
Curvularia hawaiiensis
MGI: 43.21
y = 3.3x + 5.54
R² = 0.97
y = 1.18x + 7.70
R² = 0.75
Botryotinia fuckeliana
MGI: 22.08
y = 4.04x + 2.52
R² = 0.99
y = 3.01x + 1.99
R² = 0.99
Radial growth (mm)
Time after inoculation (days)

Fig. 1

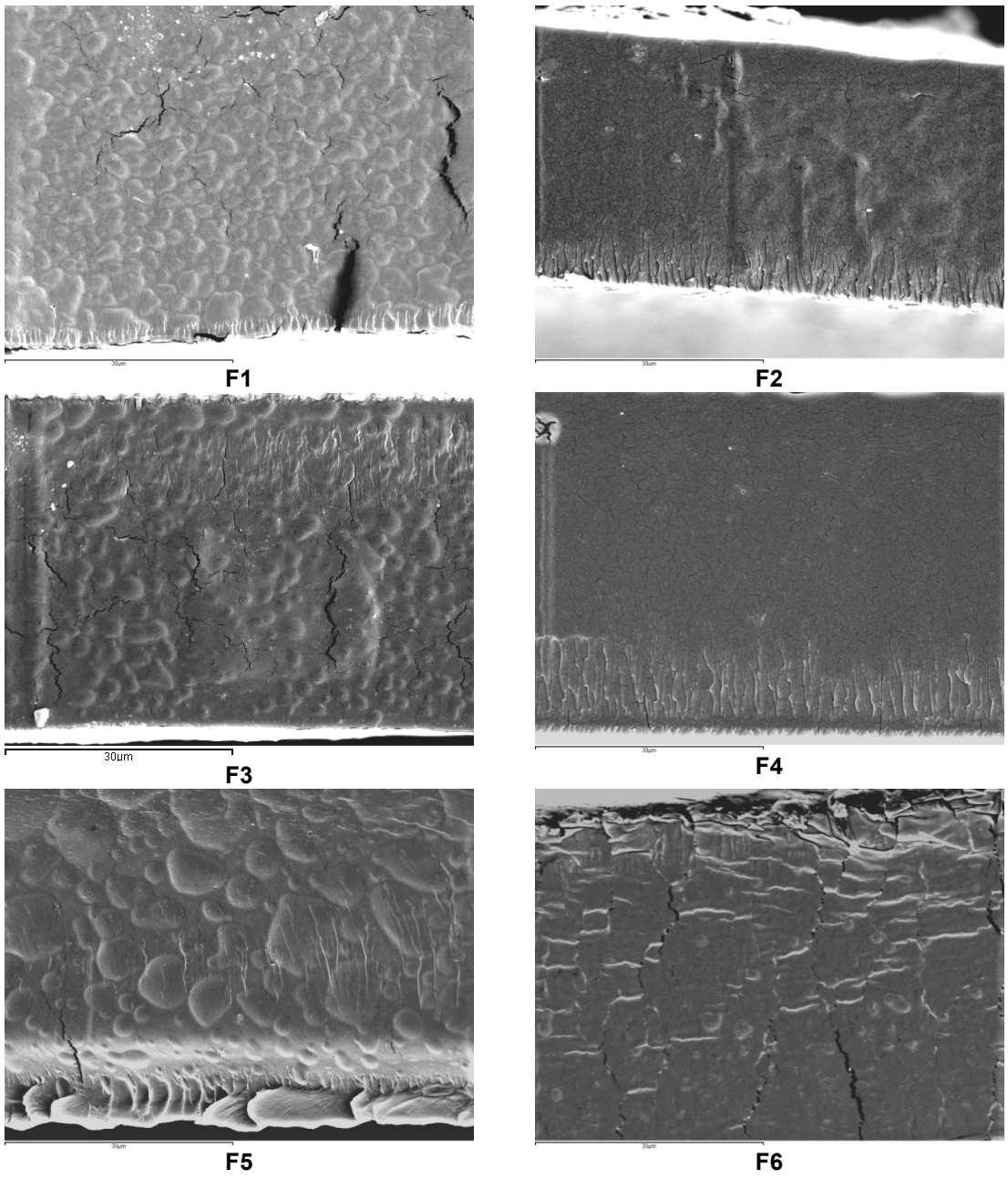


Fig. 2

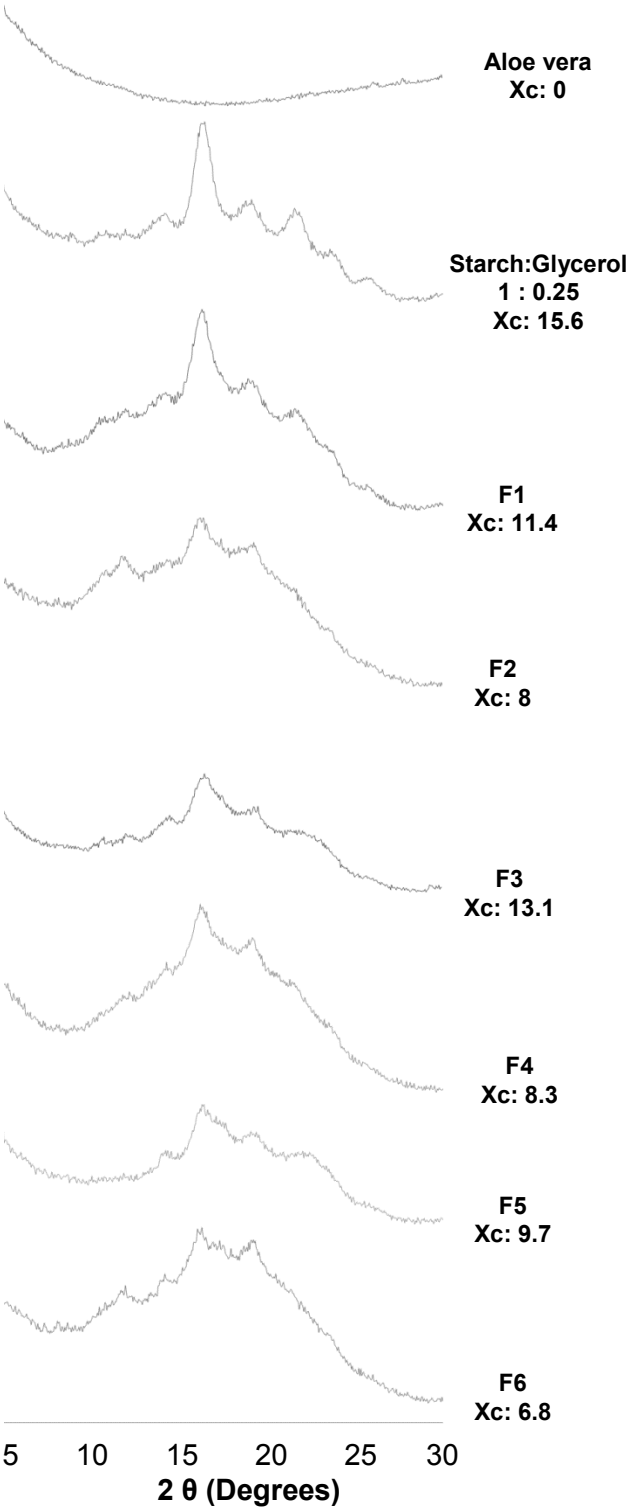
Aloe vera
Xc: 0
Starch:Glycerol
1 : 0.25
Xc: 15.6
F1
Xc: 11.4
F2
Xc: 8
F3
Xc: 13.1
F4
Xc: 8.3
F5
Xc: 9.7
F6
Xc: 6.8
5
10
15
20
25
30
2 θ (Degrees)

Fig. 3

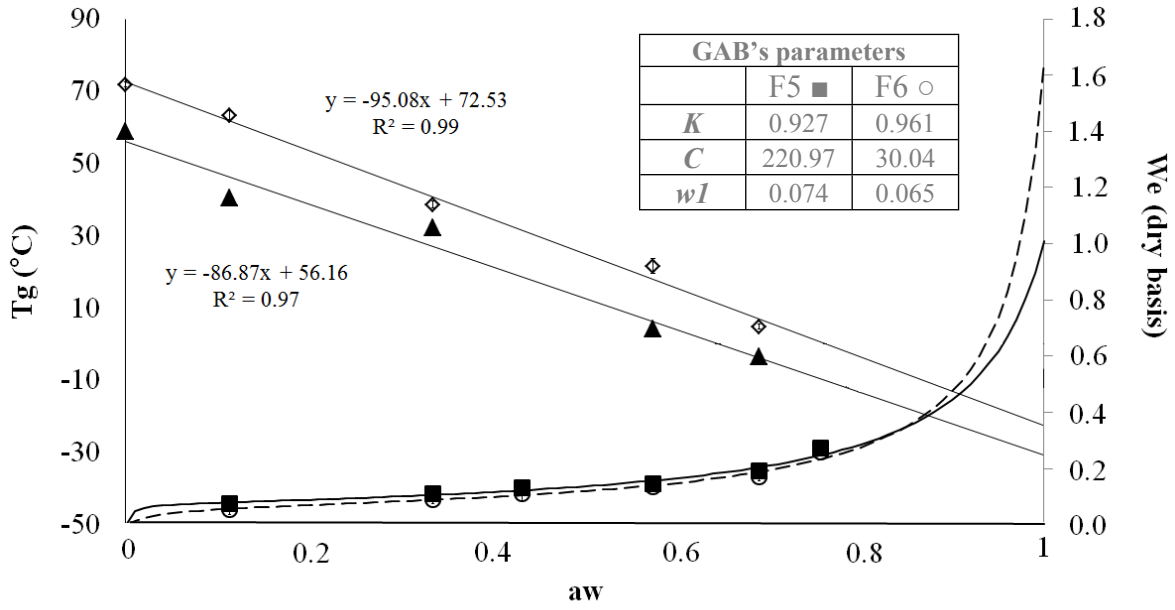
GAB's parameters
F5 ■
F6 ○
K
0.927
0.961
C
220.97
30.04
w1
0.074
0.065
y = -95.08x + 72.53
R² = 0.99
y = -86.87x + 56.16
R² = 0.97
Tg (°C)
We (dry basis)
aw
90
70
50
30
10
-10
-30
-50
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
0.2
0.4
0.6
0.8
1

Fig. 4

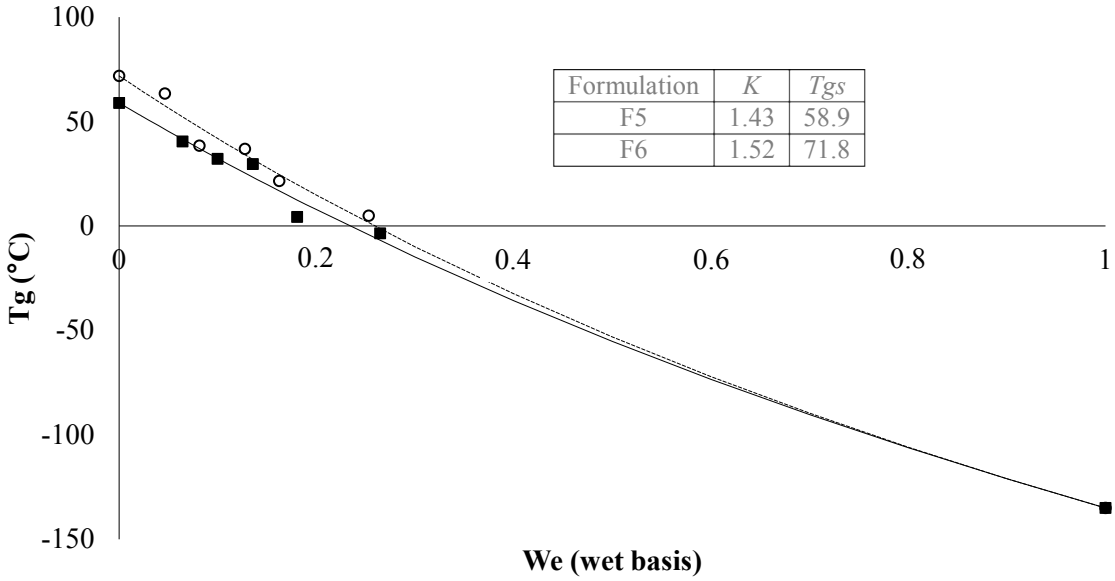
100
50
0
-50
-100
-150
0
0.2
0.4
0.6
0.8
1
Tg (°C)
We (wet basis)
Formulation | K | Tgs
F5 | 1.43 | 58.9
F6 | 1.52 | 71.8

Fig. 5

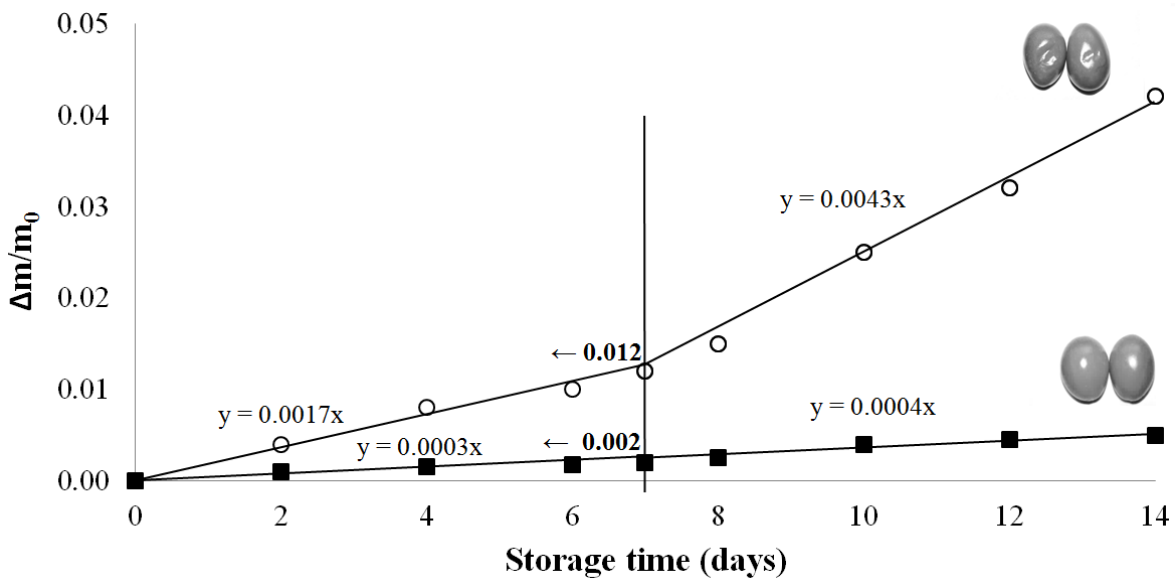
0.05
0.04
0.03
0.02
0.01
0.00
Δm/m0
y = 0.0043x
← 0.012
y = 0.0017x
y = 0.0003x
← 0.002
y = 0.0004x
0
2
4
6
8
10
12
14
Storage time (days)

Fig. 6

Figure and table captions

**Fig. 1** Radial growth of different fungi discs incubated on PDA with (■) and without (○) Aloe vera gel. Mycelium Growth Inhibition (MGI) values at 7th day of incubation at 25°C are shown.

**Fig. 2** SEM micrographs of the studied films conditiones at 75.7% RH and 10 °C

**Fig. 3** X-Ray diffraction patterns of the film formulations conditioned at 75.7% RH and 10 °C

**Fig. 4** Water sorption isotherms at 10ºC and glass transition temperature at each water activity for film formulations. Experimental points of isotherms (F5: ■ and F6: ○) and GAB fitted model (F5: ____ and F6 - - -). Experimental points of glass transition temperatures (F5: ▲ and F6: ◊) and their trend continuous lines

**Fig. 5** Glass transition temperatures of starch based films containing glycerol and Aloe vera as a function of the film moisture content. Experimental points (F5: ■ and F6: ○) and Gordon & Taylor fitted model (F5: ____ and F6 - - -)

**Fig. 6** Weight loss of cherry tomatoes with (■) and without (○) coating (F6 formulation) stored under 85% RH at 10°C (first period) and at 25 °C (second period)

**Table 1**. Mass fraction (Xi, g compound/g dry formulation) of the different components (starch: S, Aloe vera: Av and glycerol: Gly) in dry formulation. Mean values and standard deviation of thickness, water content (g water/g dried film), water vapour permeability ($g \cdot mm/KPa \cdot h \cdot m^2$), gloss (60°) and internal transmittance (IT) at 450 nm of the different films stored at 75.7% relative humidity and 10 °C.

**Table 2**. Development of cherry tomatoes, inoculated with Fusarium oxysporum, with and without preventive or curative coating (F6 formulation) stored at 85% RH at 10 °C (first 7 days) and at 25 °C (second period).

**Table 1**. Mass fraction (Xi, g compound/g dry formulation) of the different components (starch: S, Aloe vera: Av and glycerol: Gly) in dry formulation. Mean values and standard deviation of thickness, water content (g water/g dried film), water vapour permeability (g·mm/KPa·h·m2), gloss (60°) and internal transmittance (IT) at 450 nm of the different films stored at 75.7% relative humidity and 10 °C.

| Property | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| $X_s$ | 0.632 | 0.674 | 0.571 | 0.606 | 0.444 | 0.465 |
| $X_{Av}$ | 0.210 | 0.225 | 0.286 | 0.303 | 0.444 | 0.465 |
| $X_{Gly}$ | 0.158 | 0.101 | 0.143 | 0.091 | 0.112 | 0.070 |
| **Thickness (µm)** | $66 \pm 4^{a}$ | $65 \pm 5^{a}$ | $66 \pm 4^{a}$ | $66 \pm 3^{a}$ | $64 \pm 5^{a}$ | $67 \pm 5^{a}$ |
| **Water content** | $0.29 \pm 0.02^{b}$ | $0.26 \pm 0.04^{ab}$ | $0.28 \pm 0.02^{ab}$ | $0.27 \pm 0.06^{ab}$ | $0.27 \pm 0.02^{ab}$ | $0.25 \pm 0.02^{a}$ |
| **WVP** | $2.86 \pm 0.04^{c}$ | $2.18 \pm 0.08^{a}$ | $2.5 \pm 0.2^{b}$ | $2.2 \pm 0.12^{a}$ | $2.8 \pm 0.2^{bc}$ | $2.36 \pm 0.07^{b}$ |
| **Gloss** | $7 \pm 2^{a}$ | $12 \pm 2^{b}$ | $8 \pm 2^{a}$ | $14 \pm 1^{bc}$ | $12 \pm 1^{b}$ | $17 \pm 2^{c}$ |
| **IT** | $69.3 \pm 1.3^{a}$ | $81 \pm 2^{c}$ | $70.5 \pm 1.2^{a}$ | $79 \pm 2^{bc}$ | $76.4 \pm 0.8^{b}$ | $84.2 \pm 0.6^{d}$ |

Different superscript letters with in the same row indicate significant differences among formulations ($p < 0.05$).

**Table 2**. Development of cherry tomatoes, inoculated with Fusarium oxysporum, with and without preventive or curative coating (F6 formulation) stored at 85% RH at 10 °C (first 7 days) and at 25 °C (second period).

| Day | Treatment | Good appearance (%) | Soft fruit (%) | *Fusarium* presence (%) |
|---|---|---|---|---|
| **1** | Preventive treatment | 100 | 0 | 0 |
| | Curative treatment | 100 | 0 | 0 |
| | Control | 100 | 0 | 0 |
| **5** | Preventive treatment | 100 | 0 | 0 |
| | Curative treatment | 100 | 0 | 0 |
| | Control | 80 | 20 | 0 |
| **10** | Preventive treatment | 50 | 20 | 30 |
| | Curative treatment | 50 | 20 | 30 |
| | Control | 40 | 10 | 50 |
| **14** | Preventive treatment | 30 | 20 | 50 |
| | Curative treatment | 40 | 10 | 50 |
| | Control | 0 | 30 | 70 |

Highlights

- Aloe vera gel was most effective against Fusarium oxysporum
- Aloe vera could be effectively incorporated into starch matrices to obtain coatings
- Films with Aloe vera ratio were effective at controlling fungal decay in tomatoes