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DOCTORAL THESIS

Starch-based coatings with thyme essential oil for fruit preservation

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CONSIDERA: que la memoria titulada **"Starch-based coatings with thyme essential oil for fruit preservation"** que presenta **Dª Mayra Ileana Sapper** para optar al grado de Doctor por la Universitat Politècnica de València, reúne las condiciones adecuadas para constituir su tesis doctoral, por lo que **AUTORIZA** a la interesada para su presentación.

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Fdo. Amparo Chiralt Boix

Directora de Tesis

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A mi amada mamá,

A mi familia,

Y en especial a Javi.

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ABSTRACT

Starch is abundant, edible and low-cost and has a very good film-forming capacity after its gelatinisation, giving rise to transparent films with high oxygen barrier capacity. These characteristics are suitable to obtain edible films and coatings for food applications, which, in turn, can act as carriers of different functional or active compounds. However, these films or coatings present some drawbacks, such as their high water sensitivity and low water vapour barrier capacity and retrogradation during storage time. The incorporation of different components to starch formulations can mitigate these shortcomings. In this Thesis, different strategies have been analysed to tailor starch formulations for the purposes of obtaining useful coatings in postharvest fruit preservation.

Starch was partially substituted by microbial gums (xanthan (X), gellan (G), and pullulan (P)) in order to improve film functional properties. Blend starch-gum films were obtained by casting, using glycerol as plasticizer. Moisture sorption capacity and water vapour and oxygen permeability were reduced by the presence of gellan gum in the starch films. It also had a positive effect on the tensile properties, enhancing the films' strength and resistance to break and preventing retrogradation phenomena. Xanthan gum also increased the tensile strength of the starch films, but did not reduce their water sorption capacity and water vapour permeability. Functional properties were not notably improved by the addition of pullulan. Then, 10 and 20 % starch could be substituted by gellan gum to obtain films with more adequate properties for food packaging/coating purposes.

Starch-gellan (9:1 and 8:2 ratios) blend films containing thyme essential oil (EO) were also studied in order to provide antifungal activity to the formulations, given the proven activity of this EO. This was incorporated either by direct emulsification or encapsulated in lecithin liposomes. These films exhibited antifungal effect in *in vitro* tests against *Alternaria alternata* and *Botrytis cinerea*. The encapsulation of the EO in lecithin liposomes allowed for greater EO retention in the films, enhancing their antifungal activity, which were more effective against *B. cinerea* than against *A. alternata*. The antifungal action was slightly affected by the polymer matrix composition. Thus, the greatest starch ratio enhanced the fungal growth when the content of the active compound was low. All the films exhibited high oxygen barrier capacity. The presence of lecithin enhanced their water vapour barrier capacity and reduced the film stiffness, resistance to break and extensibility, giving the films a slightly brownish colour. The films with lecithin-encapsulated EO, with a starch-gellan ratio of 8:2, were the most effective at controlling fungal growth.

In order to apply these starch-gellan (8:2) formulations as fruit coatings, the surface properties of apple, tomato and persimmon, and the spreadability coefficient of these liquid formulations on the fruit surface, were analysed as a function of the concentration of Tween 85, as surfactant. The fruit skins behaved as low-energy surfaces. The values of the contact angles and surface tension of EO-free formulations were positively influenced by the addition of Tween 85. However, in the presence of emulsified or lecithin-encapsulated thyme EO, the

surfactant exerted a negative effect, depending on its concentration. Coating-forming systems containing emulsified or encapsulated EO did not require surfactant to improve their already good spreadability, while Tween 85 at $5x10^4$ mg/L notably improved this property in EO-free formulations.

Starch-gellan coatings with a ratio of 8:2, containing or not emulsified or lecithin-encapsulated EO, were applied on apples and persimmons. The addition of Tween 85 to the EO-free starch-gellan formulations, to improve their spreadability, was also evaluated in apples. Coatings did not reduce the weight loss in apples, but they prevented water loss in persimmons. In contrast, no significant effect of the coatings was observed on respiration rates and respiration quotient of persimmons, whereas they increased the respiration rates and quotient in apples. Coatings did not affect the changes in fruit firmness either in apples or persimmons. On the other hand, in the *in vivo* assays, the coatings without lecithin reduced the incidence and severity of black spot caused by *A. alternata* in persimmons, and the severity of grey mould caused by *B. cinerea* in apples. However, despite its proven antifungal action in the *in vitro* tests, the incorporation of EO did not exert an additional antifungal effect on the fruit and seemed to exert a negative effect on some other fruit quality attributes. This could be explained by the particular interactions of the coating components, fruit surface and pathogen which determine the overall effect of coatings on a given plant tissue. Neither was lecithin observed to exert a positive effect on the controlled postharvest parameters. Then, starch-gellan coatings without lecithin or thyme EO could be used in persimmons to control weight loss and reduce the incidence of infections caused by *A. alternata*.

RESUMEN

El almidón es recurso abundante, comestible y de bajo coste y tiene una muy buena capacidad filmogénica después de su gelatinización, dando lugar a películas transparentes con una alta capacidad de barrera al oxígeno. Estas características son adecuadas para obtener películas y recubrimientos comestibles para aplicaciones alimentarias que, a su vez, pueden actuar como portadores de diferentes compuestos funcionales o activos. Sin embargo, estas películas o recubrimientos presentan algunos inconvenientes, como su alta sensibilidad al agua y su baja capacidad de barrera al vapor de agua y su retrogradación a través del tiempo durante el almacenamiento. La incorporación de diferentes componentes a las formulaciones de almidón puede mitigar estas deficiencias. En esta Tesis, se han analizado diferentes estrategias para adaptar las formulaciones de almidón con el fin de obtener recubrimientos útiles en la conservación poscosecha de frutas.

El almidón se sustituyó parcialmente por gomas de origen microbiano (xantano (X), gelano (G) y pululano (P)) para mejorar las propiedades funcionales de las películas. Las películas mezcla de almidón-goma se obtuvieron por casting, utilizando glicerol como plastificante. La adición de gelano a la matriz de almidón, redujo su capacidad de adsorción de agua y la permeabilidad al vapor de agua y al oxígeno. Tuvo también un efecto positivo en las propiedades mecánicas de las películas, mejorando su resistencia a la rotura y previniendo los fenómenos de retrogradación. La goma xantana también aumentó la resistencia a la rotura de las películas de almidón, pero no redujo su capacidad de adsorción de agua y la permeabilidad al vapor de agua. Las propiedades funcionales no se mejoraron notablemente con la adición de pululano. Por lo tanto, la sustitución del almidón por un 10 o 20% de gelano, podría ser una buena estrategia para obtener películas con propiedades más adecuadas con fines de envasado/recubrimiento de alimentos.

Se analizaron las películas mezcla de almidón-gelano (en proporciones 9:1 y 8:2) con aceite esencial de tomillo (EO), con el objetivo de proporcionar actividad antifúngica a las formulaciones, dada la probada actividad antifúngica de este EO. El aceite se incorporó mediante emulsificación directa o encapsulado en liposomas de lecitina. Estas películas mostraron un efecto antifúngico en las pruebas *in vitro* contra *Alternaria alternata* y *Botrytis cinerea*. La encapsulación del EO en los liposomas de lecitina promovió una mayor retención del aceite en las películas, mejorando su actividad antifúngica, siendo éstas más efectivas contra *B. cinerea* que contra *A. alternata*. La acción antifúngica se vio ligeramente afectada por la composición de la matriz polimérica. Así, una mayor proporción de almidón en el film dio lugar a mayor crecimiento fúngico a baja concentración del compuesto activo. Todas las películas exhibieron alta capacidad de barrera al oxígeno. La presencia de lecitina mejoró la capacidad de barrera al vapor de agua y redujo la rigidez, la resistencia a la rotura y la extensibilidad de las películas, otorgando a las mismas un color ligeramente amarillo. Las películas con EO encapsulado en lecitina, con una proporción de almidón-gelano de 8:2, fueron las más efectivas para controlar el crecimiento fúngico.

Con el objetivo de aplicar las formulaciones de almidón-gelano (8:2) como recubrimientos, se analizaron las propiedades superficiales de distintas frutas, como manzana, tomate y caqui, y el coeficiente extensibilidad de estas formulaciones líquidas sobre la superficie de la frutas, en función de la concentración de Tween 85, incorporado como tensoactivo. Las pieles de las frutas evaluadas se comportaron como superficies de baja energía. La adición de Tween 85 a las formulaciones sin EO, tuvo un efecto positivo en los valores de los ángulos de contacto y la tensión superficial. Sin embargo, cuando contenían EO, emulsionado o encapsulado en liposomas de lecitina, el surfactante ejerció un efecto negativo en estas propiedades, dependiendo de su concentración. Las formulaciones de recubrimiento con EO, emulsionado o encapsulado, no requirieron surfactante para mejorar su capacidad de extensibilidad, mientras que la adición de Tween 85 a una concentración de $5x10^4$ mg/L, mejoró notablemente esta propiedad en formulaciones S:G sin EO.

Los recubrimientos a base de almidón-gelano en proporción de 8:2, con o sin EO emulsionado o encapsulado en liposomas de lecitina, fueron aplicados en manzanas y caquis. La adición de Tween 85 a la formulación de almidón-gelano sin EO, para mejorar su extensibilidad, también se evaluó en manzanas. Los recubrimientos no redujeron la pérdida de peso en las manzanas, pero evitaron la pérdida de agua en los caquis. Por el contrario, no se observó un efecto significativo de los recubrimientos en las tasas de respiración y el cociente de respiración de los caquis, mientras que las tasas y el cociente de respiración aumentaron en manzanas. Los recubrimientos no tuvieron influencia en los cambios de firmeza de las frutas, ni en manzanas ni en caquis. Por otro lado, en los ensayos *in vivo*, los recubrimientos sin lecitina redujeron la incidencia y severidad de la mancha negra causada por *A. alternata* en los caquis, y la severidad del moho gris causado por *B. cinerea* en las manzanas. Sin embargo, a pesar de su probada acción antifúngica en las pruebas *in vitro*, la incorporación del EO no ejerció un efecto antifúngico adicional en la fruta y pareció ejercer un efecto negativo en algunos de sus atributos de calidad. Esto podría explicarse por las interacciones particulares entre los componentes del recubrimiento, la superficie de la fruta y el patógeno, que determinan el efecto global de los recubrimientos en un tejido vegetal dado. Tampoco se observó un efecto positivo de la lecitina sobre los parámetros postcosecha evaluados. Así, los recubrimientos a base de almidón-gelano sin lecitina ni EO de tomillo podrían aplicarse en caquis para controlar la pérdida de peso y reducir la incidencia de la infección causada por *A. alternata*.

RESUM

El midó és un recurs abundant, comestible i de baix cost i té una molt bona capacitat de formació de pel·lícules després de la seua gelatinització, la cula cosa dona lloc a pel·lícules transparents amb una alta capacitat de barrera a l'oxigen. Estes característiques són adequades per a obtenir pel·lícules i recobriments comestibles per a aplicacions alimentàries, que, al seu torn, poden actuar com a portadors de diferents compostos funcionals o actius. No obstant això, estes pel·lícules o recobriments presenten alguns inconvenients, com la seua alta sensibilitat a l'aigua i la seua baixa capacitat de barrera al vapor d'aigua i la seua retrogradació a través del temps durant l'emmagatzemament. La incorporació de diferents components a les formulacions de midó pot mitigar estes deficiències. En esta Tesi, s'han analitzat diferents estratègies per a adaptar les formulacions de midó a fi d'obtenir recobriments útils per a la conservació postcollita de fruites.

El midó es va substituir parcialment per gomes d'origen microbià (xantano (X), gelano (G) i pululano (P)) per a millorar les propietats funcionals de les pel·lícules. Les pel·lícules mescla de midó-goma es van obtenir per càsting, utilitzant glicerol com plastificant. L'addició de gelano a les pel·lícules de midó, va reduir la seua capacitat d'adsorció d'aigua i va donar lloc a pel·lícules amb permeabilitat al vapor d'aigua i al oxigen mes baixes. També va tindre un efecte positiu en les propietats mecàniques de les pel·lícules, i va millorar la seua resistència a la ruptura i va prevenir els fenòmens de retrogradació. La goma de xantano també va augmentar la resistència a la ruptura de les pel·lícules de midó, però no va reduir la seua capacitat d'adsorció d'aigua i la permeabilitat al vapor d'aigua. Les propietats funcionals no es van millorar notablement amb l'addició de pululano. Per tant, la substitució del midó per un 10 o 20% de gelano, podria ser una bona estratègia per a obtenir pel·lícules amb propietats més adequades per al envasat/recobriment d'aliments.

S'analitzaren les pel·lícules mescla de midó-gelano (en proporcions 9:1 i 8:2) amb oli essencial de timó (EO), amb l'objectiu de proporcionar activitat antifúngica a les formulacions, donada la provada activitat antifúngica d'este EO. L'oli es va incorporar mitjançant emulsificació directa o encapsulat en liposomes de lecitina. Estes pel·lícules van mostrar un efecte antifúngic en les proves *in vitro* contra *Alternaria alternata* i *Botrytis cinerea*. L'encapsulació de l'EO en els liposomes de lecitina va promoure una major retenció de l'oli en les pel·lícules, i va millorar la seua activitat antifúngica, sent estes més efectives contra *B. cinerea* que contra *A. alternata*. L'acció antifúngica es va veure lleugerament afectada per la composició de la matriu polimèrica. En les formulacions amb una major proporció de midó amb baix contingut en compost actiu va haver un major creixement fúngic. Totes les pel·lícules van exhibir alta capacitat de barrera a l'oxigen. La presència de lecitina va millorar la capacitat de barrera al vapor d'aigua i va reduir la rigidesa, la resistència a la ruptura i l'extensibilitat de les pel·lícules, i els atorga un color lleugerament groc. Les pel·lícules amb EO encapsulat en lecitina, amb una proporció de midó-gelano 8: 2, van ser les més efectives per a controlar el creixement fúngic.

Amb l'objectiu d'aplicar les formulacions de midó-gelano (8:2) com a recobriments, es van analitzar les propietats superficials de distintes fruites, com poma, tomaca i caqui, i el coeficient d'extensibilitat d'estes formulacions líquides sobre la superfície de la fruita, en funció de la concentració de Tween 85, incorporat com tensioactiu. Les pells de les fruites avaluades es van comportar com a superfícies de baixa energia. L'addició de Tween 85 a les formulacions sense EO, va tenir un efecte positiu en els valors dels angles de contacte i la tensió superficial. No obstant això, en presència de l'EO, emulsionat o encapsulat en liposomes de lecitina, el tensioactiu va exercir un efecte negatiu en estos valors, depenent de la seua concentració. Els recobriments amb EO, emulsionat o encapsulat, no van requerir tensioactiu per a millorar la seua extensibilitat, mentre que l'addició de Tween 85 a una concentració de $5x10^4$ mg/L, va millorar notablement esta propietat en les formulacions sense EO.

Recobriments a base de midó-gelano en una proporció de 8:2, amb o sense l'agregat d'EO emulsionat o encapsulat en liposomes, van ser aplicats en pomes i caquis. L'addició de Tween 85 a les formulacions sense EO, per a millorar el seu extensibilitat, també es va avaluar en pomes. Els recobriments no van reduir la pèrdua de pes en les pomes, però van evitar la pèrdua d'aigua en els caquis. Al contrari, no es va observar un efecte significatiu dels recobriments en les taxes de respiració i el quocient de respiració dels caquis, mentre que les taxes i el quocient de respiració van augmentar en les pomes. Els recobriments no van tenir influència en els canvis de fermesa de les fruites, ni en pomes ni en caquis. D'altra banda, en els assajos *in vivo*, els recobriments sense lecitina van reduir la incidència i severitat de la taca negra causada per *A. alternata* en els caquis, i la severitat de la floridura grisa causat per *B. cinerea* en les pomes. No obstant això, a pesar de la seua provada acció antifúngica en les proves *in vitro*, la incorporació de l'EO no va exercir un efecte antifúngic addicional en la fruita i va semblar que exercia un efecte negatiu en alguns atributs de qualitat de la fruita. Açò podria explicar-se per les interaccions particulars entre els components del recobriment, la superfície de la fruita i el patogen, que determinen l'efecte global dels recobriments en un teixit vegetal determinat. Tampoc es va observar un efecte positiu de la lecitina sobre els paràmetres postcollita avaluats. Així, els recobriments a base de midó-gelano sense lecitina o EO de timó podrien aplicar-se en caquis per a controlar la pèrdua de pes i reduir la incidència de la infecció causada per *A. alternata*.

PREFACE

DISSERTATION OUTLINE

This Doctoral Thesis is structured in five sections: Introduction, Objectives, Chapters, General Discussion and Conclusions.

The INTRODUCTION section discusses the state-of-the-art concerning starch-based edible coatings used to preserve the main properties of fruits and vegetables and prevent fruit fungal decay in postharvest conditions, as well as the different factors affecting the coating efficiency, such as surface properties or the incorporation of antifungal compounds. All these aspects have been examined in a review entitled "**Starch-based coatings for preservation of fruits and vegetables**".

The OBJECTIVE section presents the general and specific objectives of the Thesis, which is focused on the development of biodegradable, edible, antifungal films and coatings based on starch for fruit preservation purposes, containing microbial gums and thyme essential oil.

The obtained results are organised in four CHAPTERS, each one corresponding to a scientific publication with the usual sections: introduction, materials and methods, results and discussion and conclusions.

Chapter 1, entitled "**Improving functional properties of cassava starch-based films by incorporating xanthan, gellan, or pullulan gums**", evaluated the effect of the partial substitution of cassava starch by xanthan, gellan, and pullulan gums on the tensile properties, barrier capacity to oxygen and water vapour and water adsorption capacity of starch-based films. The results showed that films with 10 and 20 % of starch substitution by gellan gum exhibited more adequate properties for food coating/packaging purposes, reducing the moisture sorption capacity of starch films and enhancing the film strength and resistance to break. So, this gum was used to obtain the films/coatings analysed in the subsequent studies focusing on the promotion of the coating antifungal effect.

Chapter 2, entitled "**Antifungal and functional properties of starch-gellan films containing thyme (*Thymus zygis*) essential oil**", aimed to analyse the effect of the addition of emulsified or lecithin-encapsulated thyme essential oil on the structural, mechanical, barrier and optical properties of starch-gellan blend films in ratios of 9:1 and 8:2, as well as the essential oil retention and the *in vitro* antifungal properties against *Alternaria alternata* and *Botrytis cinerea*. Of the studied formulations, 8:2 starch-gellan films with lecithin-encapsulated essential were the most effective a controlling fungal growth, while exhibiting adequate functional properties as packaging/coating material. Thus, this starch-gellan ratio was chosen to develop coating systems for fruit applications.

Chapter 3, entitled "**Wettability of starch-gellan coatings on fruits, as affected by the incorporation of essential oil and/or surfactants**", analysed the surface properties of apple, tomato and persimmon and the wettability of coating-forming systems based on starch-gellan (8:2) blends, containing, or not, emulsified or lecithin-encapsulated thyme essential oil. For this purpose, different concentrations of Tween 85 were incorporated into the coating-forming

systems in order to explore its potentially beneficial effect on the coating spreadability. The wettability of the essential oil-free coatings was notably improved with Tween 85, but formulations containing emulsified or lecithin-encapsulated essential oil did not require surfactant to improve their already good spreadability.

Chapter 4, entitled "**Antifungal starch-gellan edible coatings with thyme essential oil for the postharvest preservation of apple and persimmon**", was focused on the application of the starch-gellan (8:2) coating formulations with thyme essential oil to apples and persimmons in order to analyse their effectiveness at controlling weight loss, respiration rate, fruit firmness and fungal decay caused by *B. cinerea* and *A. alternata*. According to the results obtained in **Chapter 3**, Tween 85 was also added to the essential oil-free coating in order to ensure wettability of the fruit surface and tested on apples.

In the GENERAL DISCUSSION section, the main results obtained in the different chapters were analysed together, from a global perspective,

Finally, the most relevant CONCLUSIONS of the Thesis are shown.

DISSEMINATION OF RESULTS

INTERNATIONAL JOURNALS JCR

Published

- *Review:*

Sapper, M. and Chiralt, A. (2018). **Starch-based coatings for preservation of fruits and vegetables**. *Coatings*, *8*(5), 152.

- *Research articles:*

Sapper, M., Wilcaso, P., Santamarina, M. P., Roselló, J., and Chiralt, A. (2018). **Antifungal and functional properties of starch-gellan films containing thyme (Thymus zygis) essential oil**. *Food Control*, *92*, 505–515.

Sapper, M., Talens, P., and Chiralt, A. (2019). **Improving Functional Properties of Cassava Starch-Based Films by Incorporating Xanthan, Gellan, or Pullulan Gums**. *International Journal of Polymer Science*, (6), 1–8.

Accepted

- *Research article:*

Sapper, M., Palou, L., Pérez-Gago, M. B., Chiralt, A. **Antifungal starch-gellan edible coatings with thyme essential oil for the postharvest preservation of apple and persimmon**. *Coatings.*

Submmited

- *Research article:*

Sapper, M., and Chiralt, A. **Wettability of starch-gellan coatings on fruits, as affected by the incorporation of essential oil and/or surfactants**. *LWT - Food Science and Technology*.

COMMUNICATIONS IN INTERNATIONAL CONGRESSES

- *Poster:*

Sapper, M., Talens, P., and Chiralt, A. (2017). **Improvement of tensile properties of cassava starch films by incorporation of xanthan, gellan and pullulan gums**. *International Conference of Food Innovation FOODINNOVA*. Cesena, Italy.

Sapper, M., Wilcaso, P., Santamarina, P., Roselló, J., and Chiralt, A. (2017). **Lecithin encapsulation of thyme essential oil to enhance antifungal properties of starch-gellan films**. *31st EFFoST International Conference - Food Science and Technology Challenges for the 21st Century - Research to Progress Society*. Sitges, Spain.

Sapper, M., Pauta, D., and Chiralt, A. (2018). **Starch-gellan edible coatings with thyme essential oil for post-harvest preservation of apples**. *V National and IV International Student Congress Food Science and Technology (AVECTA)*. Valencia, Spain.

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COMMUNICATIONS IN NATIONAL CONGRESSES

- *Poster:*

Villamón, D., Sapper, M., Giné-Bordonaba, J., Chiralt, A., Palou, L., Teixidó, N., Torres, R., Pérez-Gago, M. B. (2018). **Selección de recubrimientos comestibles para extender la vida útil de manzana y pera.** *XII Simposio Nacional y X Ibérico de Maduración y Postcosecha*. Badajoz, Spain.

COMMUNICATIONS IN CIENTIFIC EVENTS

- *Oral communication:*

Sapper, M., Talens, P., and Chiralt, A. **Desarrollo de formulados antigúngicos a partir de almidón de yuca para conservación de frutas y hortalizas.** *V Encuentro de Estudiantes de Doctorado de la Universitat Politècnica de València*. Valencia, Spain (2018).

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LIST OF ACRONYMS

ASTM: American Society for Testing and Materials
BCA: Biocontrol agent
BW: Beeswax
C_{ab}*: Chroma
CFS: Coating-forming system
CH: Chitosan
DMSO: dimethyl sulfoxide
EC: Edible coating
EM: Elastic modulus
EO: Essential oil
FESEM: Field Emission Scanning Electron Microscopy
FDA: American Food and Drug Administration
FFS: Film-forming solution
F_{max}: Maximum puncture force
G: Gellan gum
GR: Growth Rate
GRAS: Generally recognized as safe
h_{ab}*: Hue
HPMC: Hydroxypropyl methyllcellulose
K: Consistency
L: Lecithin
L*: Luminosity
LSD: Least significant difference
MGI: Mycelial growth inhibition.
n: Flow behaviour indices
ɳ: Apparent viscosity
OA: Oleic cid
OP: Oxygen permeability
P: Pullulan
PDA: Potato Dextrose Agar
PS: Potassium sorbate
PTFE: Polytetrafluorethylene
R: Respiration rate
RH: Relative humidity
RQ: Respiration quotient
S: Starch
SB: Sodium benzoate
SDS: Surface density of solids
SEP: Sodium ethyl paraben
SMP: Sodium methyl paraben
SP: Sodium propionate.
T_i: Internal transmittance
TS: Tensile strength
X: Xanthan gum
W_a: Work of adhesion
W_c: Work of cohesion
WI: Whiteness Index
W_s: spreading coefficient
WVP: Water vapour permeability
WVTR: Water vapour transmission rate
%E: Percentage of elongation at break
%S: Water solubility
γ_C: Critical surface tension
γ_L: Surface tension
γ_{LV}: Liquid-vapour interfacial tension
γ_{SL}: Solid-liquid interfacial tension
γ_{SV}: Solid-vapour interfacial tension
γ_S^d: Dispersive component
γ_S^p: Polar componen

I. INTRODUCTION

1. Starch-based coatings for preservation of fruits and vegetables

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Abstract

Considerable research has focused on the control of the physiological activity of fruits and vegetables in postharvest conditions as well as microbial decay. The use of edible coatings (ECs) carrying active compounds (e.g., antimicrobials) represents an alternative preservation technology since they can modify the internal gas composition by creating a modified atmosphere through the regulation of the gas exchange (oxygen, carbon dioxide, volatiles) while also limiting water transfer. Of the edible polymers able to form coating films, starch exhibits several advantages, such as its ready availability, low cost and good filmogenic capacity, forming colourless and tasteless films with high oxygen barrier capacity. Nevertheless, starch films are highly water sensitive and exhibit limited water vapour barrier properties and mechanical resistance. Different compounds, such as plasticizers, surfactants, lipids or other polymers, have been incorporated to improve the functional properties of starch-based films/coatings. This paper reviews the starch-based ECs used to preserve the main properties of fruits and vegetables in postharvest conditions as well as the different factors affecting the coating efficiency, such as surface properties or incorporation of antifungal compounds. The great variability in the plant products requires specific studies to optimize the formulation of coating forming products.

Keywords: edible coating; starch; antifungal; postharvest; preservation; fruit; vegetable; wettability.

Introduction

Fruits and vegetables are essential in the human diet due to the health and nutritional benefits associated with their intake. However, they are products with a relatively short postharvest life, since they remain as living tissues up until the time they are used for consumption and are prone to physiological and biochemical changes, which can also have physical or pathological origins (Palou, Valencia-Chamorro, & Pérez-Gago, 2015), leading to important economic losses (Olivas & Barbosa-Cánovas, 2009; Park, 1999). Fruits and vegetables lose weight during postharvest handling and storage by transpiration, resulting in textural changes and surface shrinkage that affects their shelf life. On the other hand, softening of fruit during storage is also attributed to the deterioration of the cell wall components, mainly pectin, due to the activity of various enzymes.

Postharvest treatments with conventional synthetic waxes and/or chemical fungicides have been used for many years to control postharvest decay and extend fruit shelf life. However, the continuous application of these treatments has led to health and environmental issues, associated with chemical residues, or to the proliferation of resistant pathogenic strains. The increasing restrictions on the use of agrochemicals imposed by many countries and the growing consumer demand for high quality, minimally processed fresh food products have intensified the search for new preservation methods and technologies. The use of edible coatings (ECs) has emerged as an effective and environmentally-friendly alternative to extend their shelf life (Karaca, Pérez-Gago, Taberner, & Palou, 2014) and protect them from harmful environmental effects. Such films, applied as coatings, can create semipermeable barriers to gases and water vapour, reducing respiration and weight loss and maintaining the firmness of the fresh product while providing gloss to the coated products. In addition, coatings are able to act as carriers of a wide variety of functional ingredients, such as antimicrobials, antioxidants, anti-browning agents, nutrients or flavouring and colouring compounds (Fagundes, Palou, Monteiro, & Pérez-Gago, 2015; Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2016), enhancing food stability, quality and safety, thus promoting the coatings' functional performance beyond their barrier properties (Mariniello, Giosafatto, Di Pierro, Sorrentino, & Porta, 2010). Non-toxic antifungal compounds, incorporated in edible coatings, can prevent fungal decay, which is one of the main causes for deterioration of fruits and vegetables.

An EC is a thin layer of edible material coated directly on a food surface, applied in liquid form (film-forming solution/dispersion) on the food, usually by immersing or spraying (Kang, Kim, You, Lacroix, & Han, 2013). The film-forming solution or dispersion contains a polymeric material with filmogenic capacity (Campos, Gerschenson, & Flores, 2011). The film provides a barrier against water vapour and gases and thus lower levels of O_2 and higher levels of CO_2 inside the fruit, which helps to control the enzyme activities, contributing to maintain the firmness of the coated product during storage.

The efficiency and stability of ECs depend on their composition. Polysaccharides, including starch, cellulose, pectin, alginates, chitosan and others, are naturally occurring polymers, widely used for this purpose (Hassan, Chatha, Hussain, Zia, & Akhtar, 2017), and are compatible with a broad range of functional compounds (Mehyar, Al-Qadiri, & Swanson, 2014) whose aim is to improve their properties. Starch is a promising polysaccharide for food coating/packaging purposes, when taking into account its filmogenic capacity, ready availability and low cost. The starch world market can mainly be divided into four raw materials: corn, potato, sweet potato and cassava, although the predominant source used to obtain biodegradable plastics has been corn starch. This may be due to corn being the main source of starch produced worldwide (approximately 65%), followed by sweet potato (13%) and cassava (11%) (Luchese, Spada, & Tessaro, 2017).

Starch-based coatings are colourless and have an oil-free appearance, and can be used to increase the shelf life of fruits, vegetables and other products, although due to their hydrophilic nature, they are highly water sensitive and exhibit low water vapour barrier capacity. Other components, such as plasticizers and emulsifiers (or surfactants), may be added to the polymer matrix to improve the flexibility, extensibility and/or the stability of the polymer matrix structure. Blending (with other hydrophobic compounds to limit the hygroscopicity of starch-based materials has become an economical and versatile way to obtain new materials with better properties (Cazón, Velazquez, Ramírez, & Vázquez, 2017).

This paper reviews the starch-based ECs used to preserve the main properties of fruits and vegetables in postharvest conditions. The different methods used to determine or improve surface properties of coating materials when applied on fruits and vegetables, which greatly affect coating spreading and efficiency, have been analysed. Moreover, recent studies related with the application and/or characterization of mainly starch-based edible coatings, with and without antifungal properties to prevent fruit fungal decay, have been reviewed and their main conclusions summarized.

1.1. Requirements of the coating-forming agents to preserve fruits and vegetables

The most important functional properties of edible films and coatings are their barrier properties to water vapour and gases, compound migration, their ability for physical and mechanical protection and their impact on the product appearance (colour and gloss) (Palou et al., 2015). The loss of quality in fresh products occurring during postharvest storage is associated with the biochemical and physiological changes in the live tissue, which is greatly affected by mass transfer phenomena, including moisture or oxygen exchanges, flavour loss or undesirable odour absorption (Bonilla, Atarés, Vargas, & Chiralt, 2012; Miller, Upadhyaya, & Krochta, 1998). Likewise, great losses in post-harvested products are due to microbiological alterations, mainly fungal decay, which shorten their shelf life and increase the risk of foodborne illnesses. Then, one of the main interests in coating design is the inclusion of substances with antimicrobial activity within polymeric matrices.

How effective EC is at protecting fruits and vegetables greatly depends on the product wettability to obtain a uniformly coated surface, which is influenced both by the fruit/vegetable surface properties and by the chemical composition and structure of the coating-forming polymers: the presence of different compounds, such as plasticizers, surfactants, antimicrobials or antioxidants (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). The possible loss of these molecules during coating formation can affect the thickness of the film (Park, 1999). The EC effectiveness is also closely related with other factors, such as tensile properties. Mechanical resistance is important for two reasons: to prevent the film/coating fracture and to protect fruits and vegetables from mechanical factors and the physical damage caused by impact, pressure or vibrations during storage.

As regards the mass transfer properties of the coatings, the challenges include decreasing the water vapour permeability values in order to prevent both the moisture loss in the products (weight loss) and any changes in texture, flavour and appearance (Lin & Zhao, 2007). Both loss and gain of water are nearly always considered undesirable. The coating must also provide an adequate gas barrier (low oxygen permeability values), since this respiration process accelerates the consumption of sugars and other compounds, thus increasing the ethylene production and causing senescence (Bonilla et al., 2012). In terms of their oxygen barrier properties, starch-based coatings usually stand out compared to other coating materials such as other polysaccharides or proteins (Kramer, 2009). However, in order to prevent anaerobic respiration, moderate barriers with a certain degree of oxygen and carbon dioxide permeability are needed for the respiration of living tissues (Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 2007). In this sense, depending on the different respiration rates of the fruit or vegetable, a different minimum oxygen transfer rate may be needed to avoid unwanted metabolic changes. A sufficient gas barrier could also prevent fruits and vegetables from losing volatile flavour compounds or acquiring foreign odours. The good adherence and extensibility of the coating are key factors for the enhancement of the coating functions while also improving the appearance and attractiveness of the coated fruit or vegetable. Other considerations to take into account when formulating ECs are that some active ingredients might change the organoleptic profile of the coated product, causing undesirable odours or modifications in the functional properties. Some active compounds, such as essential oils, may cause toxicity in plant cells at high concentrations, or lose their functionality when reacting with external factors or food components (Acevedo-Fani, Soliva-Fortuny, & Martín-Belloso, 2017). Figure 1 shows the main relationships between the coating properties and the quality factors that are preserved in the fruit and vegetable. The interfacial interaction between the coating-forming agents, affected by its surface properties, and the product surface energy determine how effective the product coating is at exerting adequate protection.

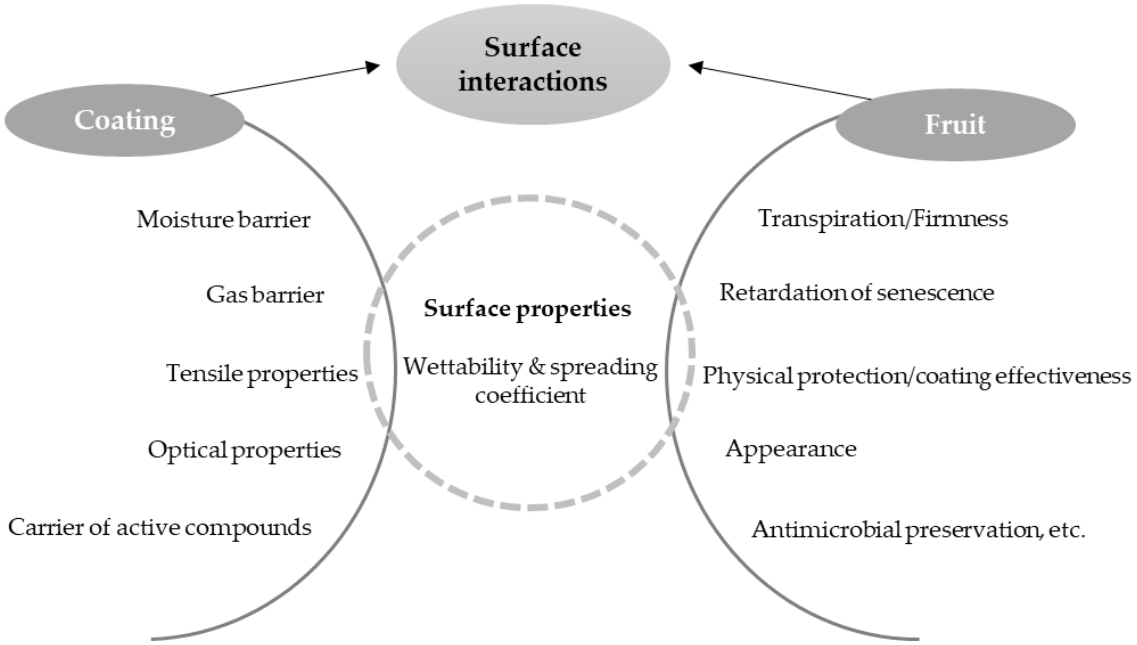

Figure 1. Relationship between coating properties and quality attributes of fruits and vegetables (Fuente: propia).

1.1.1. Factors affecting the coating spreadability

The wettability of the product by a coating solution is of particular importance, as it is crucial when defining the ability of a coating to wet and spread uniformly on the surface of the fruit or vegetable. Wettability is studied by determining the values of the spreading coefficient (W_s) as a function of the works of adhesion (W_a) (Eq. (1)) and cohesion (W_c) (Eq. (2)). The equilibrium-spreading coefficient can be defined by Equation (3) (Rulon & Robert, 1993) and can only be negative or zero. The adhesive forces promote the extension of a liquid on a solid surface and the cohesive forces its contraction. Consequently, the wetting behaviour is conditioned by the balance between these forces, and it is important to optimize the coating formulations in terms of biopolymer, plasticizer, surfactant, antimicrobial, antioxidant or other compound concentration in order to promote their spreading coefficient on a determined surface. In practical terms, the closer the W_s values are to zero, the better a surface will be coated.

$$W_a = \gamma_{LV} + \gamma_{SV} - \gamma_{SL} \quad (1)$$

$$W_c = 2 \cdot \gamma_{LV} \quad (2)$$

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{SL} \quad (3)$$

The surface tension of the coating solution, as well as its contact angle (θ) on the target solid surface can be measured and then used to determine the W_s, through the estimation of the vapour-solid-liquid interaction. The contact angle of a liquid drop on the solid surface is defined by the mechanical equilibrium established under the action of the three interfacial tensions: solid-vapour (γ_{SV}) liquid-vapour (γ_{LV}) and solid-liquid (γ_{SL}) (Eq. (4)).

Likewise, the interfacial tension can be separated into polar and dispersive components (Equation (5)) and, for pure liquids, the polar and dispersive components are known. If the surface contact angle between those liquids and the solid is obtained, the interaction can be described by Eq. (6). This can be used to estimate the dispersive (γ_S^d) and polar (γ_S^p) components of the solid surface tension if at least three pure liquids are used and the dependent variable $\left(\frac{1+\cos\theta}{2}\frac{\gamma_L}{\sqrt{\gamma_L^d}}\right)$ is plotted vs. the independent variable $\left(\sqrt{\frac{\gamma_L^p}{\gamma_L^d}}\right)$, from the intercept and slope of the fitted straight line, respectively.

Then, the determination of the solid surface energy or surface tension, which is a controlling factor in the wetting processes, involves the measurement of the contact angle of several standard liquids on the product surface in order to estimate the dispersive and polar contributions of the surface tension.

$$\cos\theta = (\gamma_{SV} - \gamma_{SL})/\gamma_{LV} \tag{4}$$

$$W_a = W_a^d + W_a^p \leftrightarrow W_a = 2\cdot\left(\sqrt{\gamma_S^d\cdot\gamma_L^d} + \sqrt{\gamma_S^p\cdot\gamma_L^p}\right) \tag{5}$$

$$\frac{1+\cos\theta}{2}\cdot\frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^p}\cdot\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_S^d} \tag{6}$$

The estimation of the critical surface tension (γ_c) can be carried out from the Zisman plots (Zisman, 1964) by plotting the cosine of the contact angle of these pure liquids against their surface tension. From the fitted straight line, the intercept with cos θ = 1 corresponds to the critical surface tension, which is an imaginary point of the γ_{SV}, frequently used to describe the wettability of the solid surface. It represents the value of γ_{LV} of the liquid above which its spreading on the solid surface is complete (Eq. (7)).

$$\gamma_C = \lim_{\gamma LV} \text{ as } \theta \to 0 \tag{7}$$

The reported values of the critical surface tension are generally lower than the solid surface tension values (Dann, 1970). In Table 1, the surface and the critical surface tension of some fruits and vegetables are summarized. All of the fruit surfaces were found to be of low-energy and had the ability to participate in non-polar interactions, as a consequence of the higher values of the dispersive component of the surface tension.

Table 1. Surface properties of fruit and vegetable skins/peels (mN/m).

Fruit/Vegetable Skin	Polar Component	Dispersive Component	Solid Surface Tension	Critical Surface Tension (γ_c)	Reference
Mango	1.71	24.77	26.48	19.5	(Lima et al., 2010)
Apple	0.68	27.13	27.81	25.4	
Apple (cv. Golden)	0.68	27.13	27.81	25.4	(Carneiro-da-Cunha et al., 2009)
Acerola	4.35	23.59	27.94	9.39	(Cerqueira, Lima, Teixeira, Moreira, & Vicente, 2009)
Cajá	2.29	27.86	30.15	23.92	
Mango	1.47	27.57	29.04	22.68	
Pitanga	3.07	23.88	26.95	13.42	
Seriguela	4.59	26.89	31.48	19.62	
Tomato	3.04	25.67	28.71	17.4	(Casariego et al., 2008)
Carrot	0.34	26.13	26.48	24.1	
Strawberry	5.95	22.99	28.94	18.84	(Ribeiro, Vicente, Teixeira, & Miranda, 2007)
“Fuji” apple	-	-	-	18.7	(Choi, Park, Ahn, Lee, & Lee, 2002)
Garlic	-	-	-	18.3	(Hershko & Nussinovitch, 1998)
Orange Grapefruit	-	-	-	23.0	(Hagenmaier & Baker, 1993)

How the composition of coating solutions and the addition of surfactants affect the wetting properties of different fruits and vegetables has been evaluated by several authors (Table 2). It has been reported that Tween 80 was effective at reducing the surface tension of different coating solutions, through a reduction in the cohesion forces, which improved the compatibility between the solution and the surface of the fruit skin, enhancing its wettability (Carneiro-da-Cunha et al., 2009; Choi et al., 2002). Casariego et al. (2008) found that increasing chitosan and glycerol concentrations in the coating solutions reduced both the W_s and W_a, but increased the W_c. On the other hand, different formulations of galactomannans and glycerol coatings showed good values of W_s when applied on different tropical fruits (Cerqueira et al., 2009). Ribeiro et al. (2007) obtained good wettability with a coating based on 2% starch and 2% sorbitol as a plasticizer applied on strawberry.

Upon drying, a coating with adequate cohesion and adhesion must be obtained, directly affecting its performance as a preservation agent (Carneiro-da-Cunha et al., 2009; Lin & Zhao, 2007). Coating integrity also is a critical factor which depends on the film flexibility, surface tension and adhesion to the food product. Plasticizer-free matrices are too brittle and rigid because of strong interactions between the polymer chains and are incompatible with irregular surfaces, such as that of some fruits (Versino, Lopez, Garcia, & Zaritzky, 2016).

Table 2. Recent studies into the effect of different components and concentrations on the surface properties of coatings and their spreading coefficient (W_s) on fruits.

Polymer Matrix	Additives/Surfactants	Fruit/Vegetable	Main Results	Reference
Chitosan (0.5, 1.0, 1.5 % w/v)	Glycerol (0.5, 1.0, 1.5 % v/v) *Aloe vera* liquid fraction (0.5 % v/v) Tween 80 (0, 0.1, 0.2 % w/v)	Blueberry	Coatings with 0.5 % (w/v) chitosan + 0.5 % (w/v) glycerol + 0.1 % (w/v) Tween 80 + 0.5 % (v/v) *A. vera* liquid fraction presented the best W_s values (close to zero), to uniformly coat blueberry surface.	(Vieira et al., 2016)
Galactomannans (seeds of *A. pavonina* and *C. pulcherrima)* (0.5, 1.0, 1.5 % w/v)	Collagen (0.5, 1.0, 1.5 % w/v) Glycerol (0, 0.5, 1.0, 1.5 % v/v)	Mango Apple	The best W_s values for mango were obtained with blends of 0.5 % of galactomannan from *A. pavonina*, 1.5 % of collagen and 1.5 % of glycerol (W_s = −29.07 mN·m^{-1}). Blends of 0.5 % of galactomannan from *C. pulcherrima*, 1.5 % of glycerol-free collagen were the best for apples (W_s = −42.79 mN·m^{-1}).	(Lima et al., 2010)
Galactomannans (seeds of *A. pavonina* and *C. pulcherrima)* (0.5, 1.0, 1.5 % w/v)	Glycerol (1.0, 1.5, 2.0 % v/v)	Acerola Cajá Mango Pitanga Seriguela	The wettability values ranged from −36 to −26 mN·m^{-1}. For the galactomannan from *A. pavonina*, the best W_s values were obtained for acerola (0.5 % galactomannan and 1.0 % glycerol) and seriguela (0.5 % of galactomannan and 1.5 % of glycerol). For mango, pitanga and cajá, coating solutions with 0.5 %, 1.0 % and 1.5 % of galactomannan exhibited good W_s values at the different concentrations of glycerol used. When the galactomannan from *C. pulcherrima* was used, the best W_s value was obtained with solutions containing 0.5 % of galactomannan, except for mango where the best W_s value was obtained with 1.5 % of galactomannan.	(Cerqueira et al., 2009)
Policaju (1.5 and 3.0 % w/v)	Sorbitol (0.4 % w/w) Tween 80 (0 and 0.1 % w/v)	Apple	The best W_s values were found with the addition of 0.1 % (w/v) Tween 80 (W_s: −29 and −26 mN·m^{-1}, for 1.5 % and 3.0 % (w/v) policaju, respectively). The addition of Tween 80 to the solution reduced the surface tension of the liquid through a reduction in the cohesion forces, thus enhancing W_s and improving the	(Carneiro-da-Cunha et al., 2009)

Polymer Matrix	Additives/Surfactants	Fruit/Vegetable	Main Results	Reference
			compatibility between the solution and the surface of the fruit skin.	
Chitosan (1.0, 1.5, 2.0 % w/v)	Glycerol (0.25 mL/g chitosan) Sorbitol (0.5 mL/g chitosan) Tween 80 (0.02–0.1 % w/v)	Tomato Carrot	The increase in the concentration of chitosan and plasticizers reduced the values of the wettability and adhesion coefficients. The optimum W_s values were experimentally obtained with solutions of 1.5 % (w/v) of chitosan and 0.1 % of Tween 80 (w/w) as surfactant agent (W_s: −23 and −30 $mN \cdot m^{-1}$, respectively, for tomato and carrot).	(Casariego et al., 2008)
Starch (2 % w/v) Carrageenan (0.3 % w/v) Chitosan (1 % w/v)	Sorbitol (2 % w/v) Glycerol (0.75 % w/v) Tween 80 (0.01–0.1 % w/v). Tween 80 (0.01–0.1 % w/v).	Strawberry	The addition of 2 % sorbitol improved the wettability of the starch coating; however, the high surface tension of the carrageenan coatings led to high contact angles. For each polysaccharide-based coating, the best wettability was obtained for the following compositions: 2 % starch and 2 % sorbitol, 0.3 % carrageenan, 0.75 % glycerol and 0.02% Tween 80 or 1 % chitosan and 0.1 % Tween 80.	(Ribeiro et al., 2007)

1.2. Starch-based edible coatings applied to fruits and vegetables

Starch, the reserve polysaccharide of most plants, can be obtained from different sources: cereals (corn, wheat or rice), legumes (pea) and tubers (cassava or potato) and is one of the most abundant natural polysaccharides used as a food hydrocolloid. This is because of its wide-ranging functionality, relatively low cost and great ability to form transparent, tasteless, odourless films, with very good oxygen barrier properties, which is very useful for food preservation purposes (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). It has a granular structure and is composed of two macromolecules: amylose and amylopectin. Amylose is a linear polymer formed by glucose units linked by α-(1,4) whereas amylopectin is a highly branched polymer of glucose units with ramifications in α-(1,6) (Brigham, 2018; Cano, Jiménez, Cháfer, Gónzalez, & Chiralt, 2014). However, due to their hydrophilicity starch-based films/coatings exhibit water solubility and poor water vapour barrier properties (Hassan et al., 2017). It has been particularly studied in the context of the postharvest preservation of a variety of fresh horticultural products, including apples, oranges, strawberries and tomatoes (García, Martino, & Zaritzky, 1998).

To better control the weight loss during postharvest handling and storage caused by transpiration, one of the alternatives is to incorporate hydrophobic substances into the coating formulation. Saberi et al. (2018) reported a moisture loss restriction in pea starch-guar gum coatings with oleic acid and shellac, as well as a decrease in the respiration rates of treated fruit. Similar observations were described by Cháfer, Sánchez-González, González-Martínez, & Chiralt (2012) in oranges coated with polysaccharide-based coatings. In the same way, different plasticizers, especially sorbitol, had a significant effect on the delay in the change of weight and firmness of tomatoes coated with a non-conventional starch (Nawab, Alam, & Hasnain, 2017).

Starch-based coating formulations (2 wt% starch) with 2 % added sorbitol exhibited good wettability properties on the surface of strawberries. However, they were more permeable to O_2, which was associated with the high concentration of plasticizer in the dry coating. In fact, plasticizers are used to decrease the intermolecular attractions between adjacent polymeric chains, which in turn facilitates the molecular mobility and diffusion of the gas molecules through the polymer network (Ribeiro et al., 2007; Sabbah et al., 2017).

A representative summary of recent studies highlighting the positive effect of active edible coatings on the shelf life of fruit and vegetables is shown in Table 3, giving an overview of other compounds commonly used to improve the properties of starch-based coatings. Different results can be obtained and these not only depend on the kind of fruit or vegetable but also on the coating composition. In general, the incorporation of lipids into starch or polysaccharide-based coatings contribute to the reduction in the amount of water lost in the coated product and can also affect the gas exchanges, depending on the lipid ratio and its physical state. Solid lipids generally offer better resistance to the mass transfer of water or gas molecules than liquid lipids (Fabra, Talens, Gavara, & Chiralt, 2012). The lipid-polymer ratio is also an

important factor, since lipids provoke interruptions in the polymer matrix which have a great impact on the coating performance. In this sense, the adequate compatibilization of lipids and polymers and the final size distribution of lipid particles in the film also affect the coating's functional properties (Perdones, Chiralt, & Vargas, 2016). As concerns starch coatings, the amylose–amylopectin ratio affected their functionality due to the different structure of the films generated with high-amylose starch, where linear amylose, with regions of helical conformation, exhibited better packed domains in the matrix that is more crystalline in nature (Cano et al., 2014). García et al. (1998) found that coatings made with high-amylose starch better preserved the weight losses and firmness of strawberries for longer periods than coatings of medium-amylose starch. Likewise, these authors observed that coatings plasticized with sorbitol exhibited a better water vapour barrier capacity than those containing glycerol. In this sense, the authors of (Sagnelli et al., 2017) report the use of amylose-only starch obtained from transgenic plants to obtain starch materials with improved properties.

In general, coatings help to better retain different active compounds incorporated into their formulation, such as antimicrobials, on the product surface for longer storage times, thus enhancing their effectiveness. Mehyar et al. (2014) observed a better retention of potassium sorbate and antifungal activity during the refrigerated storage of apple, tomato and cucumber coated with starch-based formulations.

In fresh-cut products, the effects of starch-based coatings can also be beneficial for the maintenance of quality and safety during storage. Coatings with a starch:protein ratio of 15:85, with an added 6 % (v/w) of pink pepper phenolic compounds, prevented enzymatic browning in fresh-cut apples for 12 days (Romani, Hernández, & Martins, 2018) and cassava starch coatings decreased the respiration rate, preserving the mechanical properties and colour characteristics, of fresh-cut mango that was pre-treated with citric acid (0.5 % w/v) and peracetic acid (0.05 % w/v) (Chiumarelli, Pereira, Ferrari, Sarantópoulos, & Hubinger, 2010). In general, a good performance could be achieved with starch-based coatings in different fruits or vegetables, forming edible barriers at a competitive cost. However, other components, such as plasticizers, surfactants, lipids or other more hydrophobic polymers, must be incorporated into the coating-forming formulation in order to obtain a good adherence on the target product surface, an adequate mechanical resistance of the film and optimal barrier properties against water vapour or gas. Likewise, taking into account the different physiology and surface properties of the plant products, an optimal formulation must be developed for the different products; this must include active compounds permitting the control of fungal or bacterial decay which limits the product shelf life. In this sense, new approaches must make use of compounds that are innocuous for human health and effective at controlling microbial growth or physiological processes. The following sections analyse the new tendencies in this field.

Table 3. Starch-based coatings applied to fruits and vegetables (polymer and additives concentration in the coating-forming dispersion, except when indicated with respect to the total polymer).

Polymers	Additives	Fruit/Vegetable	Properties Evaluated in Coated Product	Main Results	Reference
Pea starch (2.5 % w/v) Guar gum (0.3 % w/v)	* Glycerol (25 % w/w) * Shellac (40 % w/w) * Oleic acid (1 % w/w) Tween 20 * With respect to the polymer	Orange	Weight loss, firmness, respiration rate, ethylene production, colour, acetaldehyde and ethanol concentrations (fruit juice), peel pitting index, fruit decay, stem-end rind breakdown, overall visual acceptability and sensory evaluation.	The incorporation of lipid compounds into pea starch-guar gum coatings reduced fruit respiration rate, ethylene production, weight and firmness loss, peel pitting, and fruit decay rate index.	(Saberi et al., 2018)
Mango kernel starch (4 % w/v)	Glycerol (2 % w/v) Sorbitol (2 % w/v)	Tomato	Weight loss, firmness, total soluble solids, total titratable acidity, ascorbic acid, fruit decay and sensory evaluation.	The formulations containing sorbitol were the most effective at maintaining the overall quality of the tomato fruit during storage	(Nawab et al., 2017)
Corn starch (2 % w/w)	* Glycerol (ratio 0.15) *Aloe vera* (ratio *A. vera*:starch 1:3) * With respect to the polymer	Tomato	Fruit appearance and weight loss.	Coating retarded weight loss in the fruits stored first at 10 °C and 85 % RH for 7 days, and subsequently at 25 °C and 85 % RH for 7 days. After 14 days of storage, the tomatoes without coatings exhibited a weight loss that was 84 times greater than the coated ones.	(Ortega-Toro, Collazo-Bigliardi, Roselló, Santamarina, & Chiralt, 2017)
Cassava starch (2 % w/v)	Cinnamon essential oil (0.01 % w/v)	Guava	Weight loss, firmness, total and soluble pectin and pectin methylesterase.	The treatment with starch and essential oil reduced mass loss and better preserved greenness for the	(Botelho, Rocha, Braga, Silva, & de Abreu, 2016)

Polymers	Additives	Fruit/Vegetable	Properties Evaluated in Coated Product	Main Results	Reference
				8 days of storage, compared to the control.	
Cassava starch (2.0 % w/v) Chitosan (0.5, 1.0, 1.5, 2.0 % w/v)	Mixture of *Lippia gracilis* Schauer genotypes in an EOs solution (EOM) (0, 1.0, 2.0, 3.0 % v/v) Glycerol (0.64 % w/v) Glycerol (1.28 % w/v)	Guava	Firmness, colour, pH, titratable acidity, total soluble solids	The incorporation of 1 % or 3 % EOM into edible chitosan-cassava starch coatings delayed the ripening process, reduced browning and inhibited colour development to a greater extent than only chitosan or cassava starch coatings.	(De Aquino, Blank, & De Aquino Santana, 2015)
Gelatin (10 % w/v) Corn starch (native, waxy or modified waxy) (3.0 or 5.0 % w/w) (Starch:gelatin blends 1:1)	Sorbitol (ratio polymer: plasticiser 1:0.1)	Grape	Weight loss and sensory evaluation.	Improved appearance was observed in coated grapes after 21 storage days under refrigerated conditions, with a reduced weight loss compared to the control group. Sensory evaluation showed that coatings did not affect the acceptability scores.	(Fakhouri, Martelli, Caon, Velasco, & Mei, 2015)
Corn starch (2 % w/v) Arabic gum (2 % w/v)	Glycerol (10 % w/w) Sorbitol (10 % w/w)	Green banana	Weight loss, firmness, colour	Coated fruits lose about 30% less weight than the uncoated fruits. The coating application was effective at maintaining the firmness of banana and slowed down the ripening process.	(Razak & Lazim, 2015)
Pea starch (4 % w/v) Potato starch (4 % w/v)	Glycerol (ratio (polymer:glycerol 2:1) Potassium	Apple Tomato Cucumber	Coatings weight and thickness, KS residual surface concentration, yeast and mould count	All the coatings better retained KS concentration on the fruit surface during refrigerated storage to provide effective antifungal activity.	(Mehyar et al., 2014)

Polymers	Additives	Fruit/Vegetable	Properties Evaluated in Coated Product	Main Results	Reference
Guar gum (1 % w/v)	sorbate (KS) (1% w/v)				
Rice starch (1.0, 1.5 and 2.0 % w/v)	Glycerol (0.4, 0.5 and 0.6 mL) Coconut oil Tea leaf extract	Tomato	Weight loss, total soluble solids, titratable acidity, ascorbic acid content, colour and microbial count.	Starch coating, with added glycerol, lipids and antioxidant compounds, delayed the change in colour of tomatoes, which can be directly attributed to the antioxidant activity of the green tea extract. It was also found to form a rigid and continuous fruit coating that was able to extend the ripening period of tomatoes in storage at room temperature.	(Das, Dutta, & Mahanta, 2013)
Cassava starch (1.0, 2.0 and 3.0 % w/v)	Potassium sorbate (0, 0.05 and 0.1% w/v)	Strawberry	Firmness, colour, sensory evaluation, coating integrity and respiration rate.	Cassava starch coatings, with or without potassium sorbate, did not cause changes in strawberries' mechanical properties, colour or sensory acceptance. Coatings showed good integrity for 2% and 3% starch, reducing the strawberries' respiration rate.	(Garcia, Pereira, de Luca Sarantópoulos, & Hubinger, 2010)
Starch (2 % w/v) Carrageenan (0.3 % w/v) Chitosan (1 % w/v)	Sorbitol (2 % w/v) Glycerol (0.75 % w/v) Tween 80 (0.01– 0.1 % w/v) Calcium chloride	Strawberry	Weight loss, firmness, total soluble solids, colour and total microbial count	Starch coatings were less effective at reducing loss of firmness than chitosan and carrageenan films, which better reduced the fruit weight loss. The minimum firmness loss was obtained with carrageenan and calcium chloride coatings.	(Ribeiro et al., 2007)

Polymers	Additives	Fruit/Vegetable	Properties Evaluated in Coated Product	Main Results	Reference
Starches with medium amylose content (MAS) (corn and potato starch); Starches with high amylose content (HAS) (corn starch, genetically modified and acorn starch product (HAP) (2 % w/v)	Glycerol and sorbitol (0, 1.0 %, and 2 % w/v)	Strawberry	Coating's water vapour permeability (sliced carrots), weight loss, firmness, anthocyanin content, surface colour, reducing and total sugar content and titratable acidity, soluble, insoluble and total solids and microbiological assays.	Starch coatings with the higher amylose content reduced fruit weight losses and retained fruit firmness for longer periods than coatings with medium amylose content starches. Both sorbitol and glycerol contributed both to reducing weight loss and to maintaining texture and surface colour of fruits. Coatings with sorbitol exhibited better water vapour barrier capacity than those containing glycerol. Sorbitol at 2 % w/v was the most effective plasticizer option.	(García et al., 1998)
Rice starch/Fish protein (3 g/mL of total solids) (15/85, 50/50 and 85/15 w/w)	Pink pepper phenolic compounds (4.0, 6.0 and 8.0 % v/w) Glycerol (25 g/g of total solids w/w)	Fresh-cut apples	Colour, browning index, firmness, mass loss, total soluble solids, pH and acidity.	The starch/protein blend (15/85) with 6 % (v/w) pink pepper phenolic compounds better preserved fresh-cut apples for 12 days, especially in terms of the inhibition of enzymatic browning.	(Romani et al., 2018)
Cassava starch (1.0 % w/v)	Glycerol (1.0 % w/v)	Fresh-cut mango (Pre-treated with 0.5 % w/v citric acid and 0.05 % w/v peracetic acid)	Weight loss, respiration rate, firmness, β-carotene content, colour, sensory evaluation and microbiological assays.	Cassava starch coatings, combined with citric acid dipping, promoted a decrease in the respiration rate, the better preservation of mechanical properties and colour characteristics and great sensory acceptance. The use of glycerol in the coating formulation promoted a	(Chiumarelli et al., 2010)

Polymers	Additives	Fruit/Vegetable	Properties Evaluated in Coated Product	Main Results	Reference
				greater weight loss, impairing fruit texture and increasing carotenogenesis.	

RH: relative humidity; EO: essential oil; EOM: essential oils mixture.

1.3. Antifungal coatings for fruit preservation

During storage, fruits are often subjected to different levels of microbial decay, mainly due to phytopathogenic fungi, which usually infect the host through wounds sustained during harvesting, handling and/or processing (Boubaker et al., 2016). Fungal disease is mainly controlled chemically, but the use of synthetic fungicides is limited due to undesirable aspects, including the toxicological hazard to human health and slow degradation periods, which could lead to environmental problems (Faleiro, 2011). The negative public perception of industrially-synthesized food antimicrobials has generated interest in the use of more naturally occurring compounds.

As reported by Palou et al. (2015), there are three categories of antifungal agents that can be incorporated into ECs: (a) synthetic food preservatives or GRAS (Generally recognized as safe) compounds with antimicrobial activity, including some organic and inorganic acids and their salts (benzoates, sorbates, carbonates, propionates, etc.) and parabens (ethyl and methyl parabens) and their salts, (b) natural compounds, such as essential oils (EOs) or plant extracts (thyme oil, carvacrol, cinnamon, cinnamaldehyde, citral, eugenol, lemongrass, oregano, rosemary, etc.); and (c) antimicrobial antagonists (yeasts or bacteria).

The use of antifungal compounds, such as organic acids and various plant extracts or EOs, are among those mostly antimicrobial substances studied as a possible means of controlling the growth of phytopathogens in fruits and vegetables during postharvest shelf life (Junqueira-Gonçalves, Alarcón, & Niranjan, 2013). There are a great number of in vitro and in vivo (inoculated fruits and vegetables) studies dealing with this topic, in which different active compounds have been tested against different fungi through their direct application or incorporated into film-forming formulations. In Table 4, different studies analysing the in vitro antifungal activity of different natural extracts or essential oils are summarized. Likewise, Table 5 shows several in vitro studies on the antifungal effect of these kinds of products incorporated into different polymer matrices to obtain active films. Studies into the application of these kinds of coatings to fruits or vegetables so as to extend their shelf-life are shown in Table 6, where the main findings of the authors are remarked on.

The antifungal activity of some plant extracts, such as Aloe vera, can be attributed to the presence of bioactive compounds, such as quinones and phenol compounds (flavonoids), which can be more or less active against different fungi. Moringa plant extracts had a significant effect on the growth rate of *C. gloeosporiodes*, *A. alternata* and *L. theobromae*, a fact that can be closely linked to the high concentration of phenolic compounds in the tissue (Tesfay, Magwaza, Mbili, & Mditshwa, 2017). *B. cinerea* and *P. expansum* were better inhibited than *A. niger* in the presence of pulp or liquid fractions of *A. vera* (Vieira et al., 2016). *Aloe vera* gel was also effective at controlling the growth of several fungi, exhibiting the greatest efficacy against *F. oxysporum*. When incorporated into corn starch matrices in a *Aloe vera* solids–starch ratio of up to 1:1, using glycerol as plasticizer, these coatings were effective at controlling fungal decay in cherry tomatoes and were a natural, non-toxic alternative to synthetic fungicides (Ortega-Toro et al., 2017).

EOs extracted from plants are rich sources of bioactive compounds (terpenoids, phenolic compounds) which have been recognized as antifungal agents (Boubaker et al., 2016). In order to achieve effective antimicrobial activity, high concentrations of essential oils are generally needed. The incorporation of essential oils into biopolymer matrices (active coatings) can be a useful strategy to improve coating functionality in terms of the enhancement of the antimicrobial properties of the coatings, while reducing the matrix's hygroscopic character. Likewise, the incorporation of EOs into the coating matrix allows for a reduction in the cost and minimizes their intense aroma perception (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). (Perdones, Sánchez-González, Chiralt, & Vargas, 2012) observed an enhanced in vitro antifungal activity against *B. cinerea* of chitosan films after the incorporation of lemon essential oil, although a decrease in the inhibition capacity was reported after two days of storage. This decrease was explained by considering the changes in the availability of the antimicrobials, controlled by the rate of diffusion of the active compounds into the agar medium throughout the storage period and the progressive evaporation of volatiles.

Edible composite coatings based on hydroxypropyl methylcellulose, hydrophobic components (beeswax and shellac) and food preservatives with antifungal properties (potassium sorbate, sodium benzoate and sodium propionate), were effective at reducing the incidence of *P. digitatum* and *P. italicum* during the long-term cold storage of mandarins (Valencia-Chamorro, Pérez-Gago, Del Río, & Palou, 2010). Lemongrass essential oil has been observed to be effective as a treatment for the control of anthracnose (*C. capsici*) of bell pepper *in vitro*, due to the presence of numerous secondary compounds. This was attributed to the ability of the oil to penetrate through the cell membrane, resulting in deteriorative biological processes. However, the antimicrobial activity of the EO was higher in the in vitro tests than that observed in *in vivo* applications on bell pepper, which was attributed to the inability of the oil to adhere to the bell pepper surface (Ali, Noh, & Mustafa, 2015).

Biological control is also a promising alternative to unpopular synthetic fungicides, and research into postharvest biocontrol has increased in recent decades (Droby, Wisniewski, Macarisin, & Wilson, 2009; Marín, Atarés, & Chiralt, 2017). The main characteristics of an ideal biocontrol agent (BCA) are related to its biosafety, activity in a range of environments and against a variety of pathogens, and ease of management and use (Ruiz-Moyano et al., 2016). Extensive research has been developed to understand the mechanisms by which BCAs exert their action against pathogens. Nevertheless, in many cases, the suggested modes of action whereby antagonists wield their biocontrol effect have not been totally elucidated. Competition for nutrients and space between the pathogen and the antagonist is considered to be the main mode of action, but other mechanisms, such as parasitism or the production of secondary metabolites, have also been reported. Several microorganisms, which can act as microbial antagonists, have received considerable attention as controlling agents of diseases in fruits. Table 7 shows some examples of BCA applied to the preservation of different fruits.

The combined application of BCAs and edible coatings or films offers many possibilities, both because of the wide variety of matrices which can be used and their potential benefits for the survival and retention of the antagonists. In this sense, the coating-forming formulations should

contain components which not only allow for coating formation, but are also compatible with the cells and provide them with an adequate substrate for nutrition and growth (Marín, Atarés, & Chiralt, 2017). (Marín et al., 2016) evaluated the effect of different coating-forming systems containing *C. sake* CPA-1 as BCA, based on different biopolymers (corn starch, hydroxypropylmethylcellulose, sodium caseinate, or pea protein) with and without surfactants, on the adherence, viability and survival of *C. sake* cells, as well as on their biocontrol efficacy against *B. cinerea* infections of coated grapes. Taking into account the relative increase in the survival and efficacy of *C. sake*, and the cost of ingredients, sodium caseinate or corn starch were the most suitable coatings with which to obtain formulated biocontrol *C. sake* products. Likewise, (Marín, Atarés, Cháfer, & Chiralt, 2017) developed dried formulations of *C. sake* CPA-1 based on starch derivatives, which exhibit good stability at a very low moisture content. These formulates can be used as antifungal coating agents when applied on different fruits that are susceptible to *B. cinerea* infections, after their dispersion in water at the adequate concentration.

Table 4. Recent studies into natural antifungal compounds with potential use in fruits and vegetables.

Direct Application

Antifungal Component	Fungus Tested	Antifungal Test	Result	Reference
Moringa leaf (LE) and seed (SE) extracts (methanolic and ethanolic extraction methods)	*Colletotrichum gloeosporiodes* *Alternaria alternata* *Lasiodiplodia theobromae*	Mycelial plugs (3 mm diameter) placed on PDA plates. Evaluation: Radial growth inhibition.	Ethanolic LE and SE were less effective at reducing the GR of *C. gloeosporioides* and *A. alternata*. Methanolic extracts were the least effective against the isolates, except for *L. theobromae* which was relatively inhibited (5 %) after ten days.	(Tesfay et al., 2017)
Aloe vera gel	*Fusarium oxysporum* *Alternaria alternate* *Colletotrichum gloeosporioides* *Bipolaris spicifera* *Curvularia hawaiiensis* *Botryotinia fuckeliana*	Mycelial plugs (8 mm diameter) placed on PDA plates. Evaluation: Radial growth inhibition.	The GR of the fungi decreased when *A. vera* was present in the medium. *C. gloesporoides* showed the lowest MGI values (7 days), and the greatest inhibition occurred for *F. oxysporum.*	(Ortega-Toro et al., 2017)
Basil and thyme essential oil	*Aspergillus niger* *Botrytis cinerea* *Rhizopus stolonifer*	Spore suspension (0.1 mL of 10^4 spores/mL) on PDA plates. Filter paper circle (2.5 cm diameter) embedded with different amounts of oils (3.5, 7.0, and 14 mg/plate)	A notable inhibition halo was detected for basil and thyme oil from 14 mg of oil per plate and no effect was observed for smaller amounts.	(Perdones et al., 2016)
Aloe vera extract (pulp and liquid fractions)	*Botrytis cinerea* *Penicillium expansum* *Aspergillus niger*	Spore suspension (10^4 spores/mL sterile water), using sterile well microplates. Fungal growth was monitored spectrophotometrically at	*B. cinerea* and *P. expansum* presented greater growth inhibition in the presence of *A. vera* extracts than *A. niger.* With 0.5 % of *A. vera* liquid and pulp, an inhibition of around 80 % was observed for *B. cinerea*. With 100 % of each *A. vera*	(Vieira et al., 2016)

Antifungal Component	Fungus Tested	Antifungal Test	Result	Reference
		530 nm by measuring optical density.	fraction, growth was not observed over time (72 h).	
Lemongrass essential oil	*Colletotrichum capsici*	Mycelial plugs (2 mm diameter) placed on PDA plates. Evaluation: Radial growth inhibition.	Lemongrass essential oil was an effective treatment for the in vitro control of anthracnose.	(Ali et al., 2015)
Food preservatives: Ammonium carbonate, ammonium bicarbonate, potassium carbonate, potassium bicarbonate, potassium silicate, potassium sorbate, sodium carbonate, sodium bicarbonate, sodium acetate, sodium diacetate, sodium benzoate, sodium formate, sodium propionate, sodium methylparaben, sodium ethylparaben	*Monilinia fructicola*	Mycelial plugs (5 mm diameter) placed on PDA plates. Evaluation: Radial growth inhibition.	Almost all the agents completely inhibited the radial growth of the fungus at various concentrations. Ammonium carbonate, ammonium bicarbonate and sodium bicarbonate were the most effective agents, as they completely inhibited the mycelial growth of *M. fructicola* on PDA at all concentrations tested.	(Karaca et al., 2014)

GR: Growth rate; PDA: potato dextrose agar; MGI: mycelial growth inhibition.

Table 5. Recent studies on natural antifungal compounds incorporated into coating-forming formulations (in vitro tests) with potential use in fruits and vegetables.

Application to Coatings (*In Vitro*)

Compound	Fungus	Antifungal Test	Coating Matrix	Main Findings	Reference
Cinnamon and ginger oil	*Aspergillus niger*	Spore suspension (0.1 mL of 10^6 spores/mL) on PDA plates. Film discs (4 mm) were placed on the inoculated surfaces.	CH-CMC	Cinnamon oil was more effective than ginger oil at reducing fungal growth.	(Noshirvani et al., 2017)
Basil and thyme essential oil	*Aspergillus niger* *Botrytis cinerea* *Rhizopus stolonifer*	Spore suspension (0.1 mL of 10^4 spores/mL) on PDA plates. Contact and head space methods. Film circles (2.5 cm diameter) were placed on PDA (contact method) or fixed to the plate cover (head space method).	CH	CH films with thyme or basil essential oils did not inhibit the GR of the tested fungi.	(Perdones et al., 2016)
Chitosan Cinnamon leaf essential oil	*Aspergillus niger* *Botrytis cinerea* *Rhisopus stolonifer*	Spore suspension (0.1 mL of 10^4 spores/mL) on PDA plates.	CH	Chitosan–cinnamon leaf essential oil films exhibited antifungal activity against the tested fungi.	(Perdones, Vargas, Atarés, & Chiralt, 2014)
Lemon essential oil	*Botrytis cinerea*	Spore suspension (0.1 mL of 10^5 spores/mL) on PDA plates. Film discs with the same diameter as the Petri dishes were placed on the inoculated surfaces.	CH	CH film led to reduction in the GR of *B. cinerea*. The antifungal activity of CH films was enhanced by the addition of the essential oil.	(Perdones et al., 2012)
Cinnamon, clove and oregano essential oil	*Colletotrichum gloesporoides* *Fusarium oxysporum*	Spore suspension (5 x 10^6 spores/mL spores) on PDA plates. Film discs (2.4 cm diameter) were placed on the inoculated surfaces.	S-G	Films containing cinnamon essential oil were more effective against the *F. oxysporum* fungus, while films with clove and cinnamon essential oils were more active against the *C. gloeosporiodes*	(Acosta et al., 2016)

Compound	Fungus	Antifungal Test	Coating Matrix	Main Findings	Reference
				fungus. The films with oregano essential oil were always less effective than the other two.	
Mexican oregano, cinnamon and lemongrass essential oils	*Aspergillus niger* *Penicillium digitatum*	Spore suspension (10 µL of 10^6 spores/mL) on PDA plates. Vapor contact assay. Film discs with the same diameter as the Petri dishes were placed on the inoculated surfaces.	A, CH, S	CH films incorporated with Mexican oregano or cinnamon essential oils inhibited *A. niger* and *P. digitatum* by vapor contact at lower essential concentrations than those required for amaranth and starch edible films.	(Avila-Sosa et al., 2012)

GR: growth rate; PDA: potato dextrose agar; CH: chitosan; CMC: carboxymethyl cellulose; S: starch; G: gelatin; A: amaranth.

Table 6. Natural antifungal compounds applied to coatings (in vivo tests) to preserve fruits and vegetables.

Application to Coatings (*In Vivo*)

Compound	Fungus	Antifungal Test	Matrix	Fruit/Vegetable	Result	Reference
Moringa leaf (LE) and seed (SE) extracts	*Colletotrichum gloeosporiodes Alternaria alternata*	Inoculation in wounded fruit	CMC	Avocado	Compared to the untreated fruit, the LE and SE + 1% CMC coated avocados had lower disease incidence and severity	(Tesfay et al., 2017)
Aloe vera gel	*Fusarium oxysporum*	Inoculation of 5 µL of a 10^6 espores/mL, before coating application.	S	Cherry tomato	Coatings improved the preservation of cherry tomatoes by reducing fungal decay.	(Ortega-Toro et al., 2017)
Cinnamon essential oil	*Colletotrichum gloeosporiodes*	Non-inoculated	S	Guava	Cassava starch, associated with cinnamon EO, was effective at controlling anthracnose and preserving fruit quality. On the eighth day of storage, the fruits treated with EO were free of anthracnose and similar to the day of harvest.	(Botelho et al., 2016)
Aloe vera extract (liquid fraction)	*Botrytis cinerea*	Inoculation of 10 µL of 10^4 spores/mL, before coating application	CH	Blueberry	A fungistatic effect was observed during the storage period.	(Vieira et al., 2016)
Lemongrass essential oil	*Colletotrichum capsici*	Inoculation of 10 µL of 10^5 spores/mL, before coating application	CH	Green bell pepper	EO was found to be less effective in vivo than in vitro, but the combination of EO with CH did enhance the antimicrobial activity of the coating.	(Ali et al., 2015)

Compound	Fungus	Antifungal Test	Matrix	Fruit/Vegetable	Result	Reference
SMP, SEP, SB	*Alternaria alternata*	Inoculation of 10 µL of 10^6 spores/mL, before coating application	HPMC and BW	Cherry tomato	Antifungal coatings reduced the incidence and severity of alternaria black spot on inoculated cherry tomatoes, with the SB-based coating being the most effective.	(Fagundes et al., 2015)
Food preservatives: Ammonium carbonate, ammonium bicarbonate, potassium silicate, potassium sorbate, sodium carbonate, sodium diacetate, sodium benzoate, sodium propionate, sodium methylparaben, sodium ethylparaben	*Monilinia fructicola*	Immersion in a spore suspension (10^3 spores/mL) before coating application	HPMC and BW	Plum	Coatings containing bicarbonates and parabens reduced incidence of brown rot in plums, and potassium sorbate at 1.0 % was the most effective agent. All the tested coatings reduced severity of disease to some extent.	(Karaca et al., 2014)
Chitosan Cinnamon leaf essential oil	*Rhizopus stolonifer*	Immersion in a spore suspension (10^5 spores/mL) before coating application.	CH	Strawberry	All the coatings were effective at extending the shelf-life of cold-stored strawberries.	(Perdones et al., 2014)
Lemon essential oil	*Botrytis cinerea*	Immersion in a spore suspension (10^5 spores/mL) before coating application	CH	Strawberry	CH coatings reduced the percentage of infected strawberries as compared to non-coated ones after three storage days, especially when lemon essential oil was incorporated.	(Perdones et al., 2012)
Bergamot, thyme and tree oils	*Penicillium italicum*	Immersion in spore suspension (10^5 spores/mL).	CH	Orange	The greatest antifungal effectiveness against *P. italicum* was obtained for preventive treatments with coatings	(Cháfer et al., 2012)

Compound	Fungus	Antifungal Test	Matrix	Fruit/Vegetable	Result	Reference
		Preventive and curative assays			containing tea tree oil. Curative treatments were less effective and, in this case, the coatings with thyme oil showed the greatest antifungal activity.	
PS, SB, SP, and their mixtures	*Penicillium digitatum* *Penicillium italicum*	Inoculation of spore suspension (10^5 spores/mL) before coating application	HPMC, BW and shellac	Mandarins	All the coatings reduced the incidence of green and blue moulds after 2 weeks of cold storage and the severity of the disease after 6 weeks of storage.	(Valencia-Chamorro et al., 2010)

CMC: carboxymethylcellulose; S: starch; CH: chitosan, HPMC: hydroxypropyl methyllcellulose; BW: beeswax; SMP: sodium methyl paraben; SEP: sodium ethyl paraben; SB: sodium benzoate; PS: potassium sorbate; SP: sodium propionate.

Table 7. Representative antagonistic fungi and yeasts used as biocontrol agents for the preservation of fruits.

Biocontrol Agent	Source	Pathogen	Application	Reference
Cryptococcus podzolicus	Soil	*Penicillium expansum*	Apple	(Wang et al., 2018)
Hanseniaspora opuntiae *Metschnikowia pulcherrima*	Breva crops	*Penicillium expansum*	Cherry	(de Paiva et al., 2017)
Metschnikowia pulcherrima	Fig	*Botrytis cinerea* *Cladosporium cladosporioides* *Monilia laxa* *Penicillium expansum*	Apple Nectarine	(Ruiz-Moyano et al., 2016)
Pichia membranaefaciens	-	*Colletrotichum gloeosporioides*	Citrus	(Zhou, Zhang, & Zeng, 2016)
Trichoderma spp.	Soil	*Fusarium oxysporum*	Melon	(Gava & Pinto, 2016)
Cryptococcus laurentii	Pear	*Penicillium expansum*	Pear	(Zeng et al., 2015)
Saccharomices cerevisiae *Wickerhamomyces anomalus* *Metschnikowia pulcherrima* *Aureobasidium pullulans*	Naturally fermented olive brine Pomegranate	*Botrytis cinerea*	Grape	(Parafati, Vitale, Restuccia, & Cirvilleri, 2015)

Final remarks

Consumer demand for minimally processed, additive-free foods and products has led to the development of new packaging/coating materials with active properties. Starch-based edible films and coatings are an environmentally friendly alternative to synthetic polymers due to their low cost, availability, biodegradability and food contact properties. Starch can be used in combination with other polymers or compounds to improve the functional properties of the polymeric matrix, which can also carry active compounds to better control the product shelf-life. Edible coatings can be applied to fruits and vegetables to extend the product shelf-life, decrease water loss, slow down the colour change, pH and titratable acidity during storage and modify the internal atmosphere. Despite the significant benefits gained from using edible coatings for the purposes of extending the product shelf-life and enhancing the quality and microbial safety of fresh or minimally-processed fruits and vegetables, commercial applications are still very limited.

Knowledge of the surface interactions between coating- forming dispersions and the product skin is essential if film adhesion is to be understood and the coating performance optimized in terms of barrier or mechanical properties. Good surface wettability and the proper adhesion of the coating are required to ensure its functionality. Coating adhesion and durability are important for the preservation of food quality during storage. In order to truly receive the benefit of edible coatings on fruits and vegetables in commercial applications, the coating must adhere to the food surface during processing, storage, and transportation.

Given the variability in both the surface characteristics of the different fruits and vegetables, as well as in their physiological behaviour, further studies should be carried out optimizing the coating formulation for specific applications. Starch-based formulations have the advantage of their food contact properties since starch is edible and low cost and has good carrying properties for different actives which can protect fruits and vegetable from microbial decay or physiological disorders. Most of the studies into food applications have been conducted on a laboratory scale; thus, research into cost reduction and large-scale production is required.

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II. OBJECTIVES

The **general objective** of this Doctoral Thesis was to develop and characterise active films or coatings based on cassava starch and microbial biopolymers, containing thyme essential oil as a natural antifungal compound, for their application in fruit preservation.

For this purpose, the **specific objectives** were:

1. To analyse the effect of the partial starch substitution by three different microbial gums (xanthan, gellan, and pullulan) on the relevant physical properties of the cassava starch-based films (tensile, barrier, and water adsorption capacity), as well as the ageing behaviour, as a function of the equilibrium relative humidity.

2. To obtain and characterise antifungal starch-gellan films by incorporating thyme essential oil either in free form (direct emulsification) or encapsulated in lecithin liposomes, by analysing the structural and functional properties of the cast films, as well as their in vitro antifungal activity against *Alternaria alternata* and *Botrytis cinerea.*

3. To characterise the surface properties of apple, tomato and persimmon and to evaluate the wettability and spreading coefficient of the starch-gellan coating-forming liquids, containing, or not, emulsified or lecithin-encapsulated thyme essential oil, as affected by the addition of different concentrations of Tween 85.

4. To apply starch-gellan coatings incorporating thyme essential oil to apples and persimmons to evaluate the postharvest behaviour of coated fruit in terms of weight loss, respiration rates and mechanical properties, and the antifungal efficacy of these coatings applied as a curative treatment against *B. cinerea* in apple and *A. alternata* in persimmon.

III. CHAPTERS

CHAPTER 1. Improving functional properties of cassava starch-based films by incorporating xanthan, gellan or pullulan gums

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ABSTRACT

The effect of the partial substitution of cassava starch in edible films for 10 and 20 wt% of gellan, xanthan or pullulan gums was analysed in films obtained by casting. The tensile properties, barrier capacity to water vapour and oxygen and water sorption isotherms of the samples were analysed. The blend of starch with gellan gum was effective to reduce the moisture sorption capacity of starch films while reducing water vapour permeability, enhancing the film strength and resistance to break and preserving films against starch retrogradation throughout the storage time. Xanthan gum improved the tensile behaviour of the starch films, but did not reduce their water sorption capacity and water vapour permeability. Pullulan did not notably improved the functional properties of the starch films. Gellan gum at 10 and 20 wt% in the blend could be used to obtain starch films with more adequate properties for food packaging purposes.

1. INTRODUCTION

The growth in environmental concerns over non-biodegradable petrochemical-based plastics has been the driving force behind studies seeking to solve the problems associated with the plastic waste by developing bio-based/biodegradable polymers as more environmentally-friendly materials, which can be produced from a variety of agricultural commodities and/or of food waste products (Ghanbarzadeh, Almasi, & Entezami, 2011; Hassan, Chatha, Hussain, Zia, & Akhtar, 2017).

Due to its availability, biodegradability, renewability, film-forming ability and low cost (Chen & Lai, 2008), starch from different botanical sources (cassava, corn, wheat, rice, potato, pea and others) is one of the most promising natural polymers for packaging applications (López, Castillo, García, Villar, & Barbosa, 2015; Luchese, Spada, & Tessaro, 2017). Starch-based films are transparent (Mali, Grossmann, García, Martino, & Zaritzky, 2004), odorless, tasteless and highly impermeable to oxygen (Ortega-Toro, Collazo-Bigliardi, Talens, & Chiralt, 2016). However, they have some limitations such as their highly hydrophilic nature (water sensitivity), which negatively affects tensile and barrier properties as compared with conventional synthetic polymers (Jiménez, Fabra, Talens, & Chiralt, 2012a). Therefore, various approaches, such as blending with additives and/or other biopolymers (Arismendi et al., 2013; Flores, Costa, Yamashita, Gerschenson, & Grossmann, 2010; Kanmani & Lim, 2013; Kim, Choi, Byul Kim, & Lim, 2014; Kim, Choi, Kim, & Lim, 2014; Soares, Lima, Oliveira, Pires, & Soldi, 2005; Veiga-Santos, Oliveira, Cereda, Alves, & Scamparini, 2005) or the starch chemical modification, have been studied to improve these characteristics (Samsudin & Hani, 2017).

Polysaccharides produced by microorganisms, such as pullulan, gellan or xanthan, have found a wide range of applications in the food, pharmaceutical and other industries due to their particular functional properties. Some of these applications include their use as emulsifiers, stabilizers, gelling agents, film formers or thickening agents (Linton, Ash, & Huybrechtst, 1991). Gellan gum is a microbial polysaccharide secreted by the bacterium Sphingomonas elodea. Gellan polymer consists of a linear tetrasaccharide repeat unit composed of two molecules of D-glucose, one of D-glucuronic acid and one of L-rhamnose (Chandrasekaran & Radha, 1995; Fialho et al., 2008). Kim et al. (2014) reported that gellan gum was effective at improving the storage stability and mechanical properties of the starch films.

Xanthan gum is a high molecular weight extracellular polysaccharide produced by the bacterium Xanthomonas campestris and is one of the most important commercial microbial hydrocolloids used in the food industry as thickening agent and stabilizer (Linton et al., 1991). It is composed by a linear β-1,4 linked D-glucose chain substituted on every second glucose units by charged trisaccharide side chain with glucuronic acid residue between two mannose units. The inner mannose residue is normally acetylated at C(6), which is located at the external part of the helical conformation of the polysaccharide. About half of the terminal mannoses are linked to pyruvyl residues. Deprotonation of acetyl and pyruvyl residues at pH > 4.5, increases the negative charge density of the xanthan chain. It has been reported that xanthan influenced the mechanical parameters and moisture adsorption isotherm of cassava

starch films (Arismendi et al., 2013; Veiga-Santos et al., 2005). The xanthan gum enhanced film traction resistance but generated a less deformable matrix, which was associated to the gum and starch chains interaction through hydrogen bonds developing a more resistant network but with lower deformability due to the impossibility of polymeric chains to slide for maintaining the ductile behavior observed in the absence of gum (Arismendi et al., 2013). In accordance with Veiga-Santos et al. (2005), the interaction between the xanthan gum and the starch might avoid the occurrence of amylose:amylose interaction, inhibiting the starch retrogradation. The prevention of the amylose:amylose interaction provoked by this gum could also give rise to a more unfolded network with weaker interaction forces, resulting in films with lower elongation at break (Veiga-Santos et al., 2005). Likewise, the development of gum-starch hydrogen bonds might interfere with amylose packing, inhibiting the formation of polymer-water hydrogen bonds in the amorphous areas, thus limiting the water sorption capacity of the blend films (Arismendi et al., 2013).

On the other hand, pullulan is a neutral, linear and water soluble polysaccharide (Chen, Ren, Zhang, Tong, & Rashed, 2015; Shibata, Asahina, Teramoto, & Yosomiya, 2001), composed of maltotriose units linked by α-1,6 glucosidic bonds (Kanmani & Lim, 2013). It is produced by Aureobasidium pullulans in starch and sugar cultures. Pure pullulan films are transparent, water sensitive and mechanically weak (Shih, Daigle, & Champagne, 2011). The hydrophilic nature of pullulan, however, often makes its films sticky when exposed to high relative humidity conditions (Kim et al., 2014). Kim et al. (2014) found that tapioca starch-pullulan blend films exhibited greater mechanical strength and stability during storage under humid conditions, at the same time that the composite films were less water-soluble.

The blending of starch with small amounts of the above polysaccharides could represent a simple method that has the potential of improving the starch film properties while keeping the competitive cost of the material. Although there are several studies which analyse the effect of the partial substitution of starch by different gums on blend films, in terms of the improvement of the starch-based films functionality, few comparative analysis have been reported by using the same film obtaining process (Kim et al., 2014), in order to select the most appropriate blend to effectively reduce the water sensitivity of the starch-based materials. It is well known that differences in the polymer characteristics, as well as different processing techniques to obtain films, can affect the final properties of the material (Fakhouri et al., 2013; Moreno, Díaz, Atarés, & Chiralt, 2016). Therefore, for an adequate comparison, standardization of the raw materials and methods must be carried out. Likewise, no studies were found into the effect of the gums on the starch retrogradation phenomena, responsible for changes in the film mechanical properties throughout the storage time.

In this sense, the objective of this study was to analyse the effect of the partial starch substitution by three different microbial gums (xanthan, gellan and pullulan) on the relevant physical properties of the cassava starch-based films (tensile, barrier and water adsorption capacity), as well as the ageing behaviour of the films through the changes in the film tensile properties during storage, when equilibrated at different relative humidity.

2. MATERIALS AND METHODS

2.1 Materials

Cassava starch (S) was supplied by Quimidroga S.A. (Barcelona, Spain). The amylose content (w/w, %) of cassava starch was 10%, with an amylose: amylopectin ratio of 1:9.9 (determined by using an Amylose/Amylopectin Assay Procedure enzymatic kit, Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland). Xanthan gum (X) (high molecular weight,~10^6 Da), was supplied by EPSA (Valencia, Spain). Negatively charged, low acyl gellan gum (G) KELGOGEL F (MW 3-5x10^5 Da), was purchased from Premium Ingredients (Murcia, Spain), and pullulan (P) (MW 2x10^5 Da), was provided by Nagase GmbH (Dusseldorf, Germany). Glycerol, used as plasticizer, and P_2O_5, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $CuCl_2$, NaCl and KCl salts were provided by Panreac Química S.A. (Castellar del Vallés, Barcelona, Spain).

2.2 Preparation of films

Films were obtained by casting after the preparation of the film-forming solutions (FFS). Starch (2 % w/w) was dispersed in distilled water at 95 ˚C for 30 min, while hand stirring, as described by other authors (Amalia Cano, Jiménez, Cháfer, Gónzalez, & Chiralt, 2014; Jiménez, Fabra, Talens, & Chiralt, 2012c; Moreno et al., 2016), to induce complete starch gelatinization and cooled down to reach room temperature. This low starch concentration was selected in order to make the blending with the gum solutions easier due to their high viscosity. Glycerol was added to the aqueous gelatinized starch at 0.25 g/g of starch, on the basis of previous studies using glycerol plasticized starch-based films (Cano et al., 2015; Jiménez, Fabra, Talens, & Chiralt, 2012b). The starch-glycerol aqueous system was homogenized using a rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500 rpm for 3 min. Aqueous solutions of pullulan (2 % w/w), xanthan (1 % w/w) and gellan (2 % w/w) gums were prepared in distilled water. Gum concentration was selected on the basis of the high viscosity of the respective solutions, especially for xanthan and gellan gums. Pullulan was dissolved in water under magnetic stirring (400 rpm) at room temperature. Xanthan and gellan gums were heated while magnetically stirred (400 rpm) at 90 ˚C for 60 min until the complete dissolution. Glycerol was also added to the gum solutions (0.25 g/g polymer). The solution heating was carried out to accelerate the dissolution kinetics. For the starch-gum blends, the polymer solutions were mixed in the adequate proportion to obtain starch:gum ratios of 90:10 and 80:20. The FFSs were stirred for at least 60 min and degassed at room temperature by using a vacuum pump (MZ 2C NT, Vacuubrand GMBH + CO KG, Wertheim, Germany). Additionally, the apparent viscosity of the newly prepared FFSs at steady shear rate of 100 s^{-1} was characterised in triplicate at 25 ˚C using a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. Table 1 shows the apparent viscosity values, where the effect of each gum on the starch solution viscosity can be observed. Likewise, it shows the much higher viscosity of gellan gum due to its tendency to gelation at the used concentration.

Table 1. Mean values and standard deviation of the apparent viscosity (η at 100 s^{-1} Pa s) of the film-forming solutions with pure polymers and the different starch: gum ratios.

Sample		Starch: gum ratio	
		90:10	80:20
S	0.0118 ± 0.0003^{ab}	-	-
X	0.0838 ± 0.0006^{e}	0.0331 ± 0.0001^{c}	0.0619 ± 0.0005^{d}
G	1.62 ± 0.03^{f}	0.0220 ± 0.0001^{bc}	0.0301 ± 0.0002^{c}
P	0.00321 ± 0.00003^{a}	0.00780 ± 0.00002^{ab}	0.0083 ± 0.0001^{ab}

Different superscript letters (a - f) indicate significant differences among formulations ($p < 0.05$).

To obtain the films, a mass of the FFS containing 1.5 g of total solids was poured onto polytetrafluorethylene (PTFE) plates (15 cm diameter) and allowed to dry under natural convection for approximately 48 h at 25 ˚C and 45% relative humidity (RH). Films with 100 % starch, gellan gum and pullulan were also analysed for comparison purposes, whereas it was not possible to obtain pure xanthan gum films, since a homogeneous film could not be obtained.

2.3 Film conditioning and storage

Before characterising the films, samples were equilibrated for one week at two different relative humidities (53 and 75 %) at 25 ˚C by using oversaturated solutions of $Mg(NO_3)_2$ and NaCl, respectively. Net pullulan films showed deformations during conditioning at high RH, which complicated their handling. One part of the samples was stored under the same conditions for five weeks in order to perform the second series of analyses. Film thickness was measured using a Palmer digital micrometer (Comecta, Barcelona, Spain) to the nearest 0.001 mm. Six random positions in each film sample were considered.

2.4. Characterisation of films

2.4.1 Water vapour permeability (WV

Water vapour permeability (WVP) was determined gravimetrically at 25 ˚C and RH gradients of 53-100 % and 75-100 %, following the ASTM E96-95 gravimetric method (ASTM, 1995), applying the correction proposed by Gennadios, Weller, & Gooding (1994). Payne permeability cups, 3.5 cm in diameter (Elcometer SPRL, Hermelle/s Argenteau, Belgium), were filled with 5 mL of distilled water (100% RH). Four circular samples of each formulation (3.5 cm in diameter), were secured in the cups and placed in pre-equilibrated cabinets containing oversaturated solutions of magnesium nitrate or sodium chloride to generate 53 % or 75 % RH inside the cabinet, with a fan on the top of the cup in order to reduce the resistance to water vapour transport. The cups were weighed every 1.5 h for 24 h by using an analytical balance (ME36S, Sartorius, Germany, 0.0001 g). The slope of the weight loss vs. time curve at

stationary state was used to determine the water vapour transmission rate (WVTR) and the water vapour permeability (Vargas, Albors, Chiralt, & González-Martínez, 2009).

2.4.2 Oxygen permeability (OP)

The oxygen permeation rate of the films was determined by following the ASTM standard method D3985-05 (ASTM, 2002). Measurements were taken at 53 % RH and 25 ˚C in films previously equilibrated in the same conditions, using an Ox-Tran 1/50 system (Mocon, Minneapolis, USA). The transmission values were determined every 20 min until equilibrium was reached. The exposure area of each sample during the tests was 50 cm^2. To obtain the oxygen permeability, the film thickness was considered in every case. At least two replicates per formulation were taken into account.

2.4.3 Tensile properties

A texture analyser (Stable Micro System TA. XT plus, Haslemere, England) was used to measure the mechanical properties of the films equilibrated at 53 and 75 % RH and 25 ˚C, following the ASTM standard method D882 (ASTM, 2001). Film strips (25 mm x 100 mm) were mounted in the tensile grips (A/TG model), exposing a length of 5 cm for stretching at 50 $mm \cdot min^{-1}$, until breaking. The elastic modulus (EM), tensile strength at break (TS) and percentage of elongation at break (%E) were determined from the stress–Hencky strain curves, obtained from force–deformation data.

2.4.4 Isothermal water sorption capacity

Triplicate film samples (15-20 mg), accurately weighed, were placed in desiccators at 25 ˚C and equilibrated at different RH (or water activities: aw=RH/100) by using oversaturated solutions of $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $CuCl_2$, NaCl and KCl (% RH: 32.8, 43.2, 52.9, 67.5, 75.3 and 84.3, respectively). Samples were weighed periodically (0.00001 g precision) for 3 weeks, when equilibrium was reached. Finally, the equilibrium moisture content was determined by drying in a vacuum oven (Vacioterm-T, JP Selecta S.A., Barcelona, Spain) at 60 ˚C for 24 h and placed in desiccators containing P_2O_5 until reaching constant weight.

2.5 Statistical analysis

A statistical analysis of data was performed through analysis of variance (ANOVA) using Statgraphics Centurion XVI.II (StatPoint Technologies Inc., Warrenton, VA, USA). Fisher's least significant difference (LSD) was used at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1 Barrier properties

The water vapour permeability (WVP) of polymeric films depends on many factors, including the solubility coefficient, integrity of the film matrix, hydrophobicity, diffusion rate, thickness, polymeric chain mobility and interactions between the functional groups of polymers and the permeant. Packaging has to be able to prevent moisture and gas exchange between the environment and the product. Then, the water vapour and oxygen barrier capacity of the films is key to determining the efficiency of the material as packaging. The values of the WVP of the studied films stored for 1 week under controlled conditions (53 or 75 % RH) are shown in Table 2. As expected, the WVP of the films greatly increased for the 100-75 % RH gradient, due to the greater degree of plasticization of the material and the consequent rise in molecular mobility, promoting the mass transfer rate. In both RH conditions, starch-gellan blend films exhibited the lowest WVP values. This suggests that interactions between the negatively charged gellan chains and the linear amylose chains, through hydrogen bonds, are enough effective to reduce the possibilities of water molecules to interact with the mixed matrix, which, in turn, would reduce their solubility and permeation capacity. At 53-100 % RH, pure pullulan films had the highest WVP of all the formulations, as also reported by Kanmani & Lim (2013). On the other hand, except for the starch-gellan blends, there were no notable differences between formulations at 75-100 % RH. Probably, the high plasticization level of the polymers at these high RH conditions make differences in the polymer packing less relevant to determine the barrier capacity of the films.

The oxygen permeability (OP) values of films conditioned for 1 week at 53 % RH are shown in Table 2. The OP values of net gellan and starch-pullulan blend films were significantly higher than those of the other films. The incorporation of xanthan and gellan gums contributed to a decrease in the oxygen permeability of the films, although all the films had notably low OP values, as previously reported for starch-based films (Forssell, Lahtinen, Lahelin, & Myllärinen, 2002). The reduction in the OP values provoked by the incorporation of both negatively charged chains, gellan and xanthan, could be attributed to the promotion of the hydrogen bond interactions between the gum and amylose, as previously reported by other authors (Arismendi et al., 2013; Veiga-Santos et al., 2005), creating a more compact network where the permeation of the gas molecules would be more hindered.

Table 2. Mean values and standard deviation of water vapour (WVP) and oxygen permeability (OP) of the films conditioned at 25 ˚C and 53 and 75 % RH.

Sample	WVP x 10^{10} (g/Pa s m)		OP x 10^{13} (cm^3/m s Pa)
	53 % RH	75 % RH	53 % RH
S	7.1 ± 0.2^{c}	14.0 ± 1.0^{cd}	3.6 ± 0.2^{c}
S_{90}:X_{10}	7.1 ± 0.3^{c}	13.0 ± 1.0^{bc}	2.6 ± 0.1^{b}
S_{80}:X_{20}	6.2 ± 0.4^{ab}	13.0 ± 1.0^{b}	2.7 ± 0.1^{b}
G	9.0 ± 0.1^{d}	14.5 ± 0.5^{d}	4.0 ± 0.1^{d}
S_{90}:G_{10}	5.9 ± 0.2^{a}	11.2 ± 0.3^{a}	2.7 ± 0.1^{b}
S_{80}:G_{20}	6.6 ± 0.3^{b}	10.7 ± 0.3^{a}	2.3 ± 0.1^{a}
P	11.8 ± 0.3^{f}	-	2.7 ± 0.1^{b}
S_{90}:P_{10}	10.3 ± 0.5^{e}	13.9 ± 0.2^{bcd}	4.1 ± 0.1^{d}
S_{80}:P_{20}	10.5 ± 0.2^{e}	14.0 ± 1.0^{bcd}	4.8 ± 0.1^{e}

Different superscript letters (a - f) within the same column indicate significant differences among formulations ($p < 0.05$).

3.2 Tensile properties

The elastic modulus (EM), related to the stiffness of the material, the tensile strength (TS) and the deformation percentage (%E) at break, of the films equilibrated a different relative humidity were determined for samples conditioned for 1 and 5 weeks in order to analyse the changes associated with the film hydration and ageing. These mechanical parameters, useful for describing the film tensile behaviour, are closely related to its microstructure (McHugh & Krochta, 1994), and therefore reflect the microstructural changes occurred during storage. Table 3 shows the values of the mechanical parameters obtained for the pure polymer films at the initial and final times for both conditioning conditions (53 and 75 % RH). Films obtained from pure pullulan, equilibrated at 75 % RH, exhibited deformations along with the moisture gain which did not permit the adequate measurement of their tensile properties. In contrast, pure gellan films were the stiffest (the highest EM) and most resistant to fracture (the highest TS), regardless of the storage conditions. This mechanical behaviour of the gellan films can be attributed to the high packing capacity of the linear polymer chains, also responsible for its high gelling capacity, where interactions and hydrogen bonds can occur throughout a great extent in the chains. The increase in the moisture content of the films when equilibrated with the highest RH reduced the film strength in all cases due to the enhanced the plasticising effect of the water molecules, which promoted molecular mobility and reduced the cohesion force between chains. This effect was more intense for starch (EM was reduced by 4.5) than for gellan films (EM reduction by 1.5), but could not be quantified for pullulan films due to their deformation at the highest RH. Film moisturising also promoted the stretchability, which increased more markedly in starch films.

The film ageing behaviour could also be analysed through the changes in the tensile properties over 5 storage weeks. No significant differences in the initial and final values of the tensile parameters were observed for gellan films, whereas the starch samples became stronger and less extensible under both RH conditions. This change in the mechanical behaviour of starch films could be attributed to the retrogradation phenomenon or partial migration of plasticizer. Different authors (Cano et al., 2014; Forssell, Hulleman, Myllärinen A, Moates, & Parker, 1999; Myllärinen, 2002; Rindlav-Westling, Stading, Hermansson, & Gatenholma, 1998) reported that starch retrogradation process in starch films after gelatinization, is mainly due to the amylose recrystallization in a relatively short time period, depending on the final water content, whereas amylopectin recrystallization is a long time process. Apart from crystallisation, a progressive aggregation of polymer chains throughout time has been observed for starch films (Jiménez et al., 2012c) which provokes an increase in the matrix compactness and the consequent changes in the tensile response. In contrast, pullulan films become softer, exhibiting a notable decrease in the EM. This could be attributed to a progressive water gain in the films during storage, which progressively plasticize the polymer matrix to a greater extent, giving rise to a greater chain mobility and more extensible films (Kim et al., 2014).

Table 3. Mean values and standard deviation of tensile properties (Elastic modulus, EM; tensile strength, TS and percentage of elongation, %E) of the pure polymer films conditioned at 25 ˚C and 53 or 75 % RH for 1 (initial) or 5 (final) weeks.

			Sample		
			S	G	P
RH – 53 %	Initial	EM (MPa)	$160 \pm 50^{a,1}$	$1300 \pm 150^{c,1}$	$500 \pm 60^{b,2}$
		TS (MPa)	$10 \pm 2^{a,2}$	$80 \pm 30^{b,1}$	$9 \pm 1^{a,2}$
		E (%)	$12 \pm 1^{ab,2}$	$10 \pm 6^{a,1}$	$30 \pm 10^{b,1}$
	Final	EM (MPa)	$340 \pm 50^{b,2}$	$1140 \pm 120^{c,1}$	$52 \pm 8^{a,1}$
		TS (MPa)	$6 \pm 2^{a,1}$	$34 \pm 25^{b,1}$	$2 \pm 1^{a,1}$
		E (%)	$6 \pm 2^{a,1}$	$3 \pm 2^{a,1}$	$50 \pm 20^{b,1}$
RH – 75 %	Initial	EM (MPa)	$36 \pm 5^{a,1}$	$840 \pm 50^{b,1}$	-
		TS (MPa)	$4 \pm 1^{a,1}$	$80 \pm 13^{b,1}$	-
		E (%)	$34 \pm 2^{b,2}$	$16 \pm 5^{a,1}$	-
	Final	EM (MPa)	$55 \pm 7^{a,2}$	$1000 \pm 150^{b,1}$	-
		TS (MPa)	$4 \pm 1^{a,1}$	$81 \pm 4^{b,1}$	-
		E (%)	$15 \pm 3^{a,1}$	$15 \pm 2^{a,1}$	-

Different superscript letters (a - c) in the same row indicate significant differences among formulations ($p < 0.05$) for each parameter.

Different superscript numbers (1, 2) in the same column for each parameter and film sample indicate significant differences between the two storage times ($p < 0.05$).

Figure 1 shows the elastic modulus (EM), tensile strength (TS) and elongation at break (E) for the different starch-gum blend films. In films conditioned for 1 week, adding gums to the starch matrix provoked a strengthening effect reflected in the higher values of the EM compared to the net starch film, except for starch-pullulan blends conditioned at the highest RH. Likewise,

although the film's resistance to break (TS) was considerably increased for xanthan and gellan blends, it was reduced in pullulan mixtures, mainly in the films conditioned at the highest RH. However, the film extensibility was generally reduced in all blend films with respect to net starch samples. For the negatively charged polymers (gellan and xanthan) the strengthening effect could be associated with the gum and starch chains interaction through hydrogen bonds, developing a more resistant network but with lower deformability. The restrictions associated to bonded polymeric chains to slide during the stretching compromised the ductile behaviour observed for the gum free starch films. Pullulan also exert a reinforcing effect on the starch matrix, but this disappears when films were conditioned at high RH, which could be attributed to the high water sensitivity of pullulan, which becomes highly deformable at high RH (Kim et al., 2014). Likewise, the preferential adsorption of water molecules of this gum could limit its interactions with the starch chains at high RH conditions, thus limiting its strengthening effect, as previously observed by other authors (Kim et al., 2014).

In terms of ageing, all the blend films exhibited a decrease in EM and TS values after five weeks, in contrast with the increase observed in the net starch matrix. This indicates that blending it with these gums inhibited starch retrogradation. The observed decrease in the film strength could be attributed to the rearrangement of the polymer chains during film storage, obtaining a more open matrix with greater molecular mobility, which could be due, in part, to a progressive film hydration till the true equilibrium moisture content was reached. In the films conditioned/stored at the highest RH, the values of EM and TS also decreased during storage for xanthan and pullulan blends, but increased or did not change significantly for gellan blends. At the highest RH, starch retrogradation kinetics become faster due to the highest molecular mobility associated to the water plasticization (Forssell et al., 1999). In this sense, this phenomenon seems to occur to a certain extent in gellan blends whereas it was limited in the other blend films, where a decrease in the film strength was observed. The hydration kinetics of the films till equilibrium would be affected by the gradient of water activity (aw) between the initial dried film and the external RH; the lower the driving force (at the lowest RH), the slower the hydration rate, and by the compactness of the film structure which affect the diffusion rate of water molecules. Gellan blends, in particular, did not exhibit EM or TS reduction during storage when conditioned at the highest RH, which could indicate that no changes in the film moisture content and the consequent plasticization level occurred during storage, whereas the starch retrogradation effect was prevalent in these conditions with high molecular mobility.

As concerns extensibility, gum blending provoked a reduction with respect to the net starch films, although this was less noticeable when conditioned at 53 % RH. As commented on in the next section, the gums probably limit the water sorption capacity of the films, so reducing the water plasticising effect. This would be more noticeable at the highest RH (75%), when water plasticisation was highly intense in net starch films, as previously commented on. Likewise, the water partition between the polymers, according to their relative water affinity could also affect the plasticization level of the starch matrix, this affecting its mechanical behaviour.

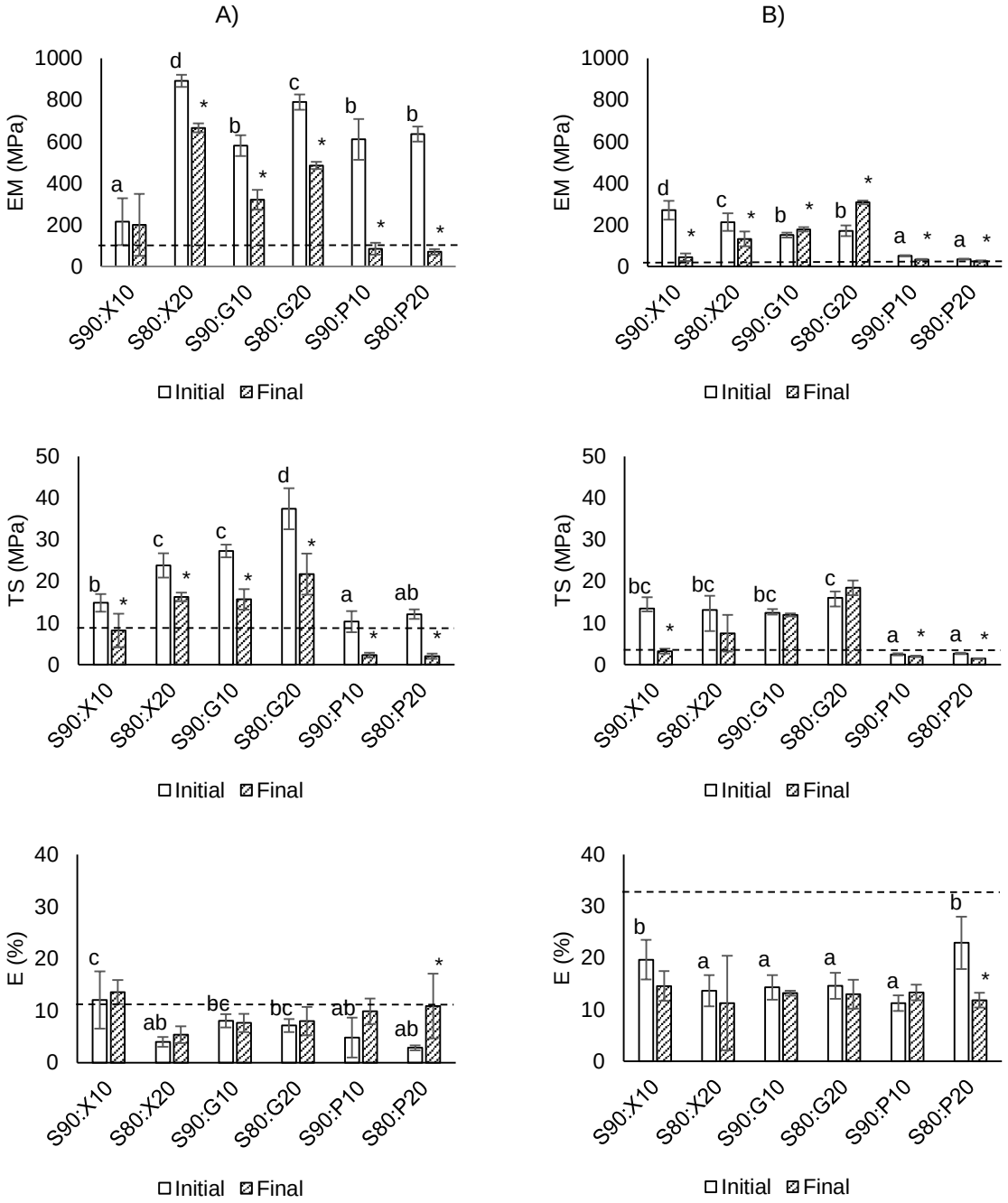

Figure 1. Elastic modulus, EM; tensile strength, TS and percentage of elongation, %E values and LSD intervals (95 % confidence level) of starch-gum blend films conditioned at: A) 53 % RH or B) 75 % RH, for 1 (Initial) or 5 weeks (Final). Different superscript letters (a - d) indicate significant differences among formulations ($p < 0.05$) for each parameter after 1 week of storage. Significant differences among formulations ($p < 0.05$) after 5 weeks of storage are indicated (*). The horizontal dashed line indicates the respective value of net starch films (conditioned for 1 week).

3.3. Isothermal water sorption capacity of the films

Table 4 shows the equilibrium moisture content (g/100 g solids) obtained for pure and blend films conditioned under different RH (between 32.8 and 84.3 %) at 25 ˚C, in order to compare the water binding capacity of the different blends and pure polymer films. At low RH pure gellan films exhibited similar water sorption capacity to the starch films, whereas pure pullulan films are slightly less hygroscopic. However from intermediate RH water binding capacity of pure gellan overcame that of starch, whereas pullulan exhibit similar or lower values than starch. The different water interactions with polar groups of the polymer chains determine the water binding capacity of the matrices depending on the equilibrium relative humidity. As concerns the blend films, the interactions between gums and starch polymers led to different effects on water sorption capacity of the blend films. Contrary to what occurs with xanthan blend films, the gellan or pullulan blends with starch exhibited lower water sorption capacity than the corresponding films with pure polymers. This could be attributed to the establishment of more efficient hydrogen bonds between the chains of the different polymers, which could reduce the number of active points for water sorption. Then, the incorporation of gellan and pullulan gums led to a decrease in the water sorption capacity of the starch films, which, in turn, will reduce their water sensitivity, making them more suitable for food packaging purposes.

Table 4. Equilibrium moisture content (g water/100 g solids) for the different film formulations conditioned at different relative humidity (RH) or a_w (RH/100).

Sample	RH (%)					
	32.8	43.2	52.9	67.5	75.3	84.3
S	8.2 ± 0.1^{c}	11.3 ± 0.5^{cd}	12.5 ± 1.3^{c}	21.4 ± 1.0^{de}	26.2 ± 0.3^{c}	39.8 ± 1.5^{d}
S_{90}:X_{10}	11.2 ± 1.0^{e}	12.3 ± 0.6^{d}	14.9 ± 1.0^{d}	22.2 ± 0.7^{e}	26.4 ± 0.4^{cd}	37.1 ± 3.6^{cd}
S_{80}:X_{20}	10.0 ± 0.3^{d}	11.3 ± 0.9^{cd}	14.8 ± 0.7^{d}	18.2 ± 1.3^{bc}	26.4 ± 0.4^{cd}	35.6 ± 1.4^{bc}
G	8.8 ± 0.4^{c}	10.5 ± 0.1^{c}	11.3 ± 1.5^{bc}	26.3 ± 1.6^{f}	29.9 ± 0.3^{e}	47.2 ± 101^{e}
S_{90}:G_{10}	7.2 ± 0.2^{b}	7.7 ± 0.8^{ab}	9.0 ± 0.5^{a}	17.9 ± 1.0^{b}	26.1 ± 0.5^{c}	33.4 ± 1.2^{b}
S_{80}:G_{20}	6.3 ± 0.4^{a}	7.9 ± 1.5^{ab}	8.7 ± 1.0^{a}	17.3 ± 1.5^{ab}	27.7 ± 1.6^{d}	35.3 ± 1.8^{bc}
P	6.5 ± 0.3^{ab}	8.5 ± 0.3^{b}	9.7 ± 0.2^{ab}	19.8 ± 0.2^{cd}	22.2 ± 1.0^{b}	39.4 ± 1.2^{d}
S_{90}:P_{10}	6.1 ± 0.1^{a}	7.2 ± 0.1^{a}	8.2 ± 0.4^{a}	16.9 ± 0.7^{ab}	19.1 ± 0.7^{a}	29.9 ± 0.4^{a}
S_{80}:P_{20}	5.9 ± 0.1^{a}	6.7 ± 0.4^{a}	9.0 ± 1.2^{a}	15.6 ± 0.4^{a}	19.2 ± 0.5^{a}	28.5 ± 1.1^{a}

Different superscript letters (a - f) within the same column indicate significant differences among formulations ($p < 0.05$).

4. CONCLUSIONS

Blending starch with gellan gum was effective at reducing the moisture sorption capacity of starch films and also reduced the water vapour and oxygen permeability and increased the film strength and resistance to break, without markedly limiting the film stretchability. Likewise, gellan-starch blends preserve films against the phenomenon of retrogradation. Xanthan gum was effective at increasing the tensile strength of the starch films but did not reduce their water sorption capacity and water vapour permeability. Pullulan was not so effective at enhancing the barrier capacity of starch films or increasing tensile strength. From the obtained results, starch substitution in the films by gellan gum at both 10 and 20 wt% is the one that most improved the barrier and tensile properties of the films and can be used to obtain films that are more useful for food packaging purposes. Particularly, foods such as nuts or snacks with low oxygen pressure requirements to ovoid oxidations, and without free water to avoid film moisturising, may be successfully packaged, allowing to extend their self-life.

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CHAPTER 2. Antifungal and functional properties of starch-gellan films containing thyme (*Thymus zygis*) essential oil

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ABSTRACT

Films based on starch-gellan blends at 9:1 and 8:2 ratios containing emulsified or lecithin encapsulated thyme (*Thymus zygis*) essential oil (EO) (0.25 or 0.5 g/g polymer), were obtained by casting method and characterized as to their structural, functional (mechanical, barrier and optical) and *in vitro* antifungal properties against *Alternaria alternata* (AA) and *Botrytis cinerea* (BC). The EO retention during the film formation was also quantified. Lecithin encapsulation of the EO allowed for greater oil retention (45-55%), which enhanced the antifungal activity of the films, which were more effective against BC than AA. All films exhibited high oxygen barrier capacity, while lecithin improved the films water barrier properties and gloss, conferring them with a slightly brownish color. Lecithin also reduced the film stiffness and resistance to break and extensibility. Of the studied formulations, 8:2 S:G films with lecithin-encapsulated EO were very effective at controlling fungal growth, while exhibiting adequate functional properties as packaging/coating materials.

Keywords: cassava starch; gellan; thyme essential oil; lecithin encapsulation; antifungal activity.

1. INTRODUCTION

Over the last few years, research into packaging has paid greater attention to biodegradable materials to substitute, at least partially, conventional plastics. Of the packaging films made from polysaccharides, those obtained from starch from different sources (corn, cassava, wheat and others) (Luchese, Spada, & Tessaro, 2017) are the most studied of the bio-based polymers, since starch is renewable, inexpensive and widely available and has good film-forming properties (Souza, Goto, Mainardi, Coelho, & Tadini, 2013). Starch films are transparent, tasteless and odorless and have good oxygen barrier properties. However, these films exhibit some drawbacks, such as the high water sensitivity and retrogradation phenomena, both giving rise to changes in the film barrier and mechanical properties during storage (Cano, Jiménez, Cháfer, Gónzalez, & Chiralt, 2014). Cassava starch has been extensively used to produce films, and, in order to improve their physical and functional properties, its blending with other biopolymers, hydrophobic substances and/or antimicrobial compounds has been proposed (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015; Ghanbarzadeh, Almasi, & Entezami, 2011; Parra, Tadini, Ponce, & Lugão, 2004).

Some gums, such as gellan, also exhibit good film-forming properties, their films being clear and insoluble in cold water (Nieto, 2009). It is an exocellular polysaccharide secreted from the bacterium *Sphingomonas elodea*, and consists of repeating tetrasaccharide units of glucose, glucuronic acid and rhamnose residues joined in a linear chain: [→3)-b-D-glucose-(1→4)-b-D-glucuronic acid-(1→4)-b-D-glucose (1→4)-a-L-rhamnose-(1→]$_n$ (Chandrasekaran & Radha, 1995; Fialho et al., 2008; Yang, Paulson, & Nickerson, 2010). Xiao et al. (2011) found that composite starch-gellan films presented improved mechanical and barrier properties, and of various gums tested, 20 % starch substitution by gellan gum appeared to be the most effective at improving the mechanical properties and storage stability of the starch films (Kim, Choi, Kim, & Lim, 2014).

Food and packaging industries are paying more and more attention to antimicrobial films and coatings due to consumer demand for minimally processed and preservative-free products. The greatest food losses can be mainly attributed to microbiological alterations, which shorten their shelf life and increase the risk of foodborne illnesses. Then, one of the main interests in active packaging design is the inclusion of substances with antimicrobial and/or antioxidant activity within polymeric matrices. Most of this interest is focused on compounds obtained from natural sources, such as essential oils (EOs), which are Generally Recognised as Safe (GRAS) by the US Food and Drug Administration (Atarés & Chiralt, 2016; Calo, Crandall, O'Bryan, & Ricke, 2015).

The antibacterial activity of different phenol-rich EOs, such as thyme EO, has been reported by several authors (Espitia et al., 2014; Jouki, Mortazavi, Yazdi, & Koocheki, 2014; Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, & Fernández-López, 2013), but fewer studies analyze the antifungal effect of these compounds (Boubaker et al., 2016; Perdones, Chiralt, & Vargas, 2016; Santamarina, Ibáñez, et al., 2016; Santamarina, Roselló, Giménez, & Blázquez, 2016)

However, on top of their potential sensory impact on the coated or packaged product, the inclusion of essential oils in packaging materials has many limitations as they can evaporate or degrade during the film formation due to either the high temperatures in thermoprocessed films or the steam drag evaporation in film casting processes, during the drying step. The encapsulation of essential oils could be a solution to maintain their usefulness for a longer time, through the control release of the compounds. One option to encapsulate hydrophobic compounds in an aqueous dispersion is the use of amphiphilic substances, such as lecithin, which can entrap the compound in liposome structures. Zhang et al. (2012) obtained stable lecithin nanoliposomes by sonication for their incorporation in chitosan films. There have been different studies on the encapsulation of volatile compounds into lecithin nanoliposomes before film preparation in order to mitigate both their losses and their sensory impact. The incorporation of lecithin liposomes containing eugenol or cinnamon leaf essential oil into chitosan films allowed for a high retention ratio of volatile compounds (Valencia-Sullca et al., 2016). Jiménez, Sánchez-González, Desobry, Chiralt, & Tehrany (2014) obtained starch-sodium caseinate films containing encapsulated orange essential oil and limonene.

The aim of this study was to obtain and characterize antifungal starch-gellan films by incorporating thyme (*Thymus zygis*) essential oil either in free form (direct emulsification) or encapsulated in lecithin nanoliposomes, by analyzing the structural and functional properties of the cast films, as well as their antifungal activity against *Alternaria alternata* and *Botrytis cinerea.*

2. MATERIALS AND METHODS

2.1. Materials and reagents

To prepare the films, cassava starch (S) (Quimidroga S.A., Barcelona, Spain), gellan gum (G) (Kelcogel F, Premium Ingredients, Murcia, Spain), non-GMO soy lecithin with 45% phosphatidylcholine (L) (Lipoid P45, Lipoid GmbH, Ludwigshafen, Germany), thyme (*Thymus zygis*) essential oil (Plantis, Artesanía Agrícola SA, Barcelona, Spain) (EO) and glycerol (Panreac Química S.A., Barcelona, Spain) were used. P_2O_5 and $Mg(NO_3)_2$ salts and UV-grade ethanol were also supplied by Panreac Química S.A. (Barcelona, Spain).

2.2. Preparation of liposome dispersions

Liposomes were obtained following the method proposed by (Valencia-Sullca et al., 2016). Lecithin was dispersed in water (5 % w/w) and stirred for at least 4 h at 700 rpm. Thyme essential oil (2.5 or 5 % w/w) was incorporated into the lecithin dispersion and two formulations (L and L-EO) were obtained, by using a sonicator (Vibra Cell, Sonics & Materials, Inc. USA) at 20 kHz for 10 min with pulses of 1 s.

2.3. Preparation of film-forming dispersions and films

Films were produced by means of casting, using cassava starch (S) and gellan gum (G) in ratios of 9:1 and 8:2, with glycerol (Gly) as plasticizer (ratio polymer: glycerol 1: 0.25). The glycerol ratio was chosen on the basis of previous studies using glycerol plasticized starch-based films (Cano et al., 2015; Jiménez, Fabra, Talens, & Chiralt, 2012). Thyme essential oil (EO) was added as an antifungal compound in a proportion of 0.25 and 0.5 g of EO/g of polymer in two different forms: by direct emulsification or encapsulated in lecithin liposomes (ratio polymer: lecithin 1: 0.5). The EO and lecithin ratios were established on the basis of previous studies into films containing essential oils, considering the potential losses of the compounds during the film drying step (Acosta et al., 2016; Valencia-Sullca et al., 2016).

For this purpose, S (2 % w/w) was dispersed in distilled water and kept at 95 °C for 30 min to induce gelatinization, and G dispersion (2 % w/w) was obtained under stirring at 90 °C for 60 min. Afterwards, glycerol was added. The S and G dispersions were mixed in adequate proportions to obtain the dispersions without EO. EO was incorporated, either by direct emulsification or encapsulated in lecithin liposomes. In the first case, the EO was added directly and the dispersions were homogenized for 3 min at 13,500 rpm using a rotor-stator homogenizer (Ultraturrax Yellow Line DL 25 Basic, IKA, Staufen, Germany). For the active dispersion with liposomes, this dispersion was added directly to the initial polymer blend and kept under magnetic stirring for 2 h. A control formulation was also obtained with lecithin liposomes without EO, as a control film. All of the dispersions were degassed by using a vacuum pump (MZ 2C NT, Vacuubrand GmbH + CO KG, Germany).

The following formulations were obtained: starch:gellan (9:1 and 8:2); controls with lecithin (9:1-L and 8:2-L); films with EO, in free form (9:1-EO and 8:2-EO), encapsulated in liposomes (9:1-EO-L and 8:2-EO-L), taking into account the two amounts of EO per g of polymer: 0.25 and 0.5. Table 1 shows the different film formulations and their respective solid compositions.

The mass of the formulations containing 1.5 g of total solids was spread evenly onto Teflon plates of 150 mm in diameter. The films were dried for approximately 48 h at 25 ºC and 45% relative humidity (RH). Dry films could be peeled intact from the casting surface and conditioned for 1 week at 53 % RH by using a saturated solution of $Mg(NO_3)_2$ at 25 ºC, prior to characterization.

2.4. Microstructural and physical properties of films

2.4.1. Microstructure and EO retention in the films

For the microstructural analysis, the film samples were conditioned in desiccators containing P_2O_5 in order to eliminate the water content; then, the films were immersed in liquid nitrogen to obtain cryofractured cross sections (Valencia-Sullca et al., 2016). All of the samples were mounted on copper stubs and platinum coated. Images were obtained by Field Emission Scanning Electron Microscopy (FESEM) (ZEISS®, model ULTRA 55, Germany), using an accelerating voltage of 2 kV.

To quantify the retention of the active compound during film formation, a known mass of dried film was placed in glass bottles containing 15 mL of an aqueous solution of UV-grade absolute ethanol 50% (v/v), and kept under stirring at 300 rpm for 24 h at 25 °C. Subsequently, aliquots of the samples were extracted and the absorbance (A) was measured at 275 nm, using a spectrophotometer (Evolution 201 UV-Vis, Thermo Fisher Scientific Inc.), as previously described by Tampau, González-Martinez, & Chiralt (2017). The oil concentration in the films was determined by means of a standard curve obtained with the EO solutions in the same solvent containing between 10 and 120 µg/mL. Blanks for the A measurements were the extract of the corresponding film without EO. The equation obtained for the standard curve was C (µg/mL) = 114.88 A.

The film thickness was measured with a digital electronic micrometer (Palmer, COMECTA, Barcelona, Spain) to the nearest 0.001 mm at six random positions.

2.4.2. Tensile properties

A universal test Machine (TA.XT plus model, Stable Micro Systems, Haslemere, England) was used to obtain the tensile stress-Henky strain curves, from which the elastic modulus (EM), tensile strength (TS) and elongation at break point (%E) of the films were determined, following ASTM standard method D882 (ASTM, 2001). Film samples (2.5 cm x 10 cm), conditioned at 25 ºC and 53 % RH for 1 week, were evaluated. Samples were mounted in the film-extension

grips of the testing machine and stretched at 50 mm min^{-1} until breaking. At least six replicates were obtained for each sample.

2.4.3. Barrier properties

The water vapor permeability (WVP) of the films was determined following a modification of the E96-95 gravimetric method (ASTM, 1995), exposing the films to a 53-100 % RH gradient at 25 ºC. To this end, 5 mL of distilled water (100% RH) were placed in Payne permeability cups (3.5 cm in diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium), put into pre-equilibrated cabinets containing saturated solutions of $Mg(NO_3)_2$ to generate 53% RH inside the cabinet and with a fan on the top of the cup in order to reduce the resistance to water vapor transport. The permeability measurements were taken by weighing the cups periodically (every 1.5 h for 24 h). Eq. (1), proposed by McHugh, Avena-Bustillos, & Krochta (1993), was used to calculate the vapor pressure on the film's inner surface (p_2).

$$WVTR = \frac{P \cdot D \cdot Ln\left[\frac{(P - p_2)}{(P - p_1)}\right]}{R \cdot T \cdot \Delta z} \quad (1)$$

where P, total pressure (atm); D, diffusivity of water through air at 25 ºC ($m^2\ s^{-1}$); R, gas law constant ($82.057 \times 10^{-3}\ m^3\ atm\ kmol^{-1}\ K^{-1}$); T, absolute temperature (K); Δz, mean stagnant air gap height (m), considering the initial and final z value; p_1, water vapor pressure on the solution's surface (atm); and p_2, corrected water vapor pressure on the film's inner surface (atm). Water vapor permeance was calculated using Eq. (2) as a function of p_2 and p_3 (pressure on the film's outer surface in the cabinet). WVP of films was obtained by multiplying permeance by film thickness.

$$WVP = \frac{WVTR}{p_2 - p_3} . thickness \quad (2)$$

The oxygen permeability (OP) was analyzed in film samples (50 cm^2) by using an Ox-Tran system (Mocon, Minneapolis, USA), following the standard method D3985-05 (ASTM, 2002) at 53 % RH and 25 ºC. The films were exposed to pure nitrogen flow on one side and pure oxygen flow on the other side. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between the two sides of the film and multiplying it by the average film thickness. Each film formulation was analyzed in triplicate.

2.4.4. Moisture content and water solubility

The moisture content of the films, equilibrated at 53 % RH and 25 ºC, was analyzed by using a gravimetric method. The film samples were first dried in a vacuum oven at 60 ˚C (Vacioterm-T, JP Selecta S.A., Barcelona, Spain) for 24 h and afterwards equilibrated in a desiccator containing P_2O_5 to remove any residual moisture. Each film formulation was analyzed in triplicate.

To determine the film's water solubility, a modification of the methodology proposed by Núñez-Flores et al. (2012) was applied. The film samples (3 cm x 3 cm), previously conditioned in P_2O_5, were weighed (m_o), immersed in glass containers with 10 mL of distilled water (m_w) and kept at 25 ºC for 24 h. Afterwards, the samples were filtered and an aliquot of the filtrate was dried at 60 °C for 24 h to constant weight in order to determine the mass ratio of soluble solids per g of water of the filtrate (m_{ss}). The solubility of the films (g soluble solids/100 g dry film) was calculated from the soluble solid content by using Eq. (3).

$$\% S = \frac{(m_{ss} \cdot m_w)}{m_o} \cdot 100 \quad (3)$$

2.4.5. Optical properties

The opacity of the films was determined by applying the Kubelka−Munk theory for multiple scattering. A spectrocolorimeter (CM-3600d Minolta Co., Tokyo, Japan) was used to obtain the reflection spectra of the films on a white (R) and black (R_0) background between 400 and 700 nm, as well as the spectrum of the white background used (R_g). From these spectra, the internal transmittance (T_i, a transparency indicator) and R_∞ (the reflectance of an infinitely thick film) were calculated using Eqs. (4 to 7)

$$T_i = \sqrt{(a + R_0)^2} - b^2 \quad (4)$$

$$a = \frac{1}{2}\left[R + \frac{R_0 - R + R_g}{R_0 \cdot R_g}\right] \quad (5)$$

$$b = (a^2 - 1)^{1/2} \quad (6)$$

$$R_\infty = a - b \quad (7)$$

Three measurements were taken from each film and three films were considered per formulation. From the R_∞ spectra, the CIE *L*a*b** color coordinates were determined using the 10º observer and the D65 illuminant as reference. Moreover, hue (hab*) and chroma (Cab*) were calculated by using:

$$h^*_{ab} = arctg\left(\frac{b^*}{a^*}\right) \quad (8)$$

$$C^*_{ab} = \sqrt{(a^{*2} + b^{*2})} \quad (9)$$

Gloss was measured using a gloss meter Multi Gloss 268 (Minolta, Langenhagen, Germany) at a 60º angle of incidence following ASTM D523 standard method (ASTM, 1999). The film samples were placed on a matte black surface, and nine measurements per formulation were taken on the side of the film that was exposed to the atmosphere during drying.

2.5. Antifungal tests

For the *in vitro* assays, stock cultures of *Alternaria alternata* (AA) CECT 20923 and *Botrytis cinerea* (BC) CECT 2100, were supplied by the Colección Española de Cultivo Tipo (CECT, Burjassot, Spain). These were preserved frozen in Agar Potato Dextrose (PDA, Scharlab, Barcelona, Spain), then incubated at 25 ºC until sporulation, and used after 7 days of active growth.

The films' antifungal properties against AA and BC were determined in Petri dishes (90 mm x 15 mm or 150 mm x 20 mm) containing Potato Dextrose Agar (PDA) growth medium. At least four replicates were obtained for each film formulation. These were inoculated with an 8 mm diameter disc of 7-day old colony on PDA of each fungus and coated using film discs 24 mm in diameter. The plates were incubated in the dark at 25 ºC for 7 days. The fungal growth was evaluated by measuring the diameter of the colonies in two perpendicular directions daily. The measurements were corrected with the initial radius of the inoculated colony (4 mm). From the radial growth vs. time curves, the growth rate (slope) and total growth inhibition for a determined time (intercept) were estimated. Mycelial growth inhibition (MGI) was also calculated after 7 days of incubation, using Eq. (10).

$$MGI = \frac{DC - DO}{DC} \times 100 \quad (10)$$

where DC is the average diameter of the colonies in the respective control plates (without EO); DO, the average diameter of colonies in the plates containing the active films (with EO).

2.6. Statistical analysis

The statistical analyses of the results were performed through an analysis of variance (ANOVA) and simple linear regression analyses done using Statgraphics Centurion XVI software (Manugistics Corp., Rockville, Md.). Fisher's least significant difference (LSD) procedure was used at the 95 % confidence level.

3. RESULTS AND DISCUSSION

3.1. Microstructure and EO final retention in the films

The functional properties of the films, such as tensile, barrier and optical properties, are directly related to their microstructure and are affected by the interactions between the film components and the drying conditions (Acosta et al., 2016; Song, Zuo, & Chen, 2018). Fig. 1 shows the FESEM images corresponding to the cross-sections of the studied films. S:G blend films, especially in a ratio of 9:1, revealed two layers of differing morphologies which evidenced the partial polymer compatibility and phase separation during the film drying step: one gellan-rich phase (lower in density) on the top and another phase, where starch predominates, at the bottom. The greater viscosity of S:G in a ratio of 8:2 mitigated the phase separation and more homogeneous films were obtained. In films containing lecithin (S:G-L), a multilayer structure was obtained by the formation of lecithin lamellar structure, with the loss of the vesicular structure, during the drying step due to the lipotropic mesomorphism of polar lipids (Krog, 1990; Larsson & Dejmek, 1990). These kinds of layered films, when amphiphilic compounds were incorporated into polymer films, were previously observed for different fatty acids in sodium caseinate films (Fabra, Jimenez, Atares, Talens, & Chiralt, 2009; Fabra, Pérez-Masiá, Talens, & Chiralt, 2011).

When the free EO was incorporated at the lowest concentration, no visible drops of oil were observed in the films, which may be attributed to the great losses during film drying, only retaining a small amount bound to the polymer chains. However, big droplets appear in films containing the highest amount of free EO, thus revealing a greater EO retention in the films. On the other hand, when the EO is incorporated as liposomes, lecithin seems to better maintain its vesicular structure, probably due to the interactions of the EO compounds with the liposomal associations, promoting EO retention in the dried films. Bigger lipid particles could be observed for the highest EO proportion, probably due to the greater progression of the destabilization phenomena (flocculation and coalescence) in the film-forming aqueous emulsions during the film drying step when the EO content increased.

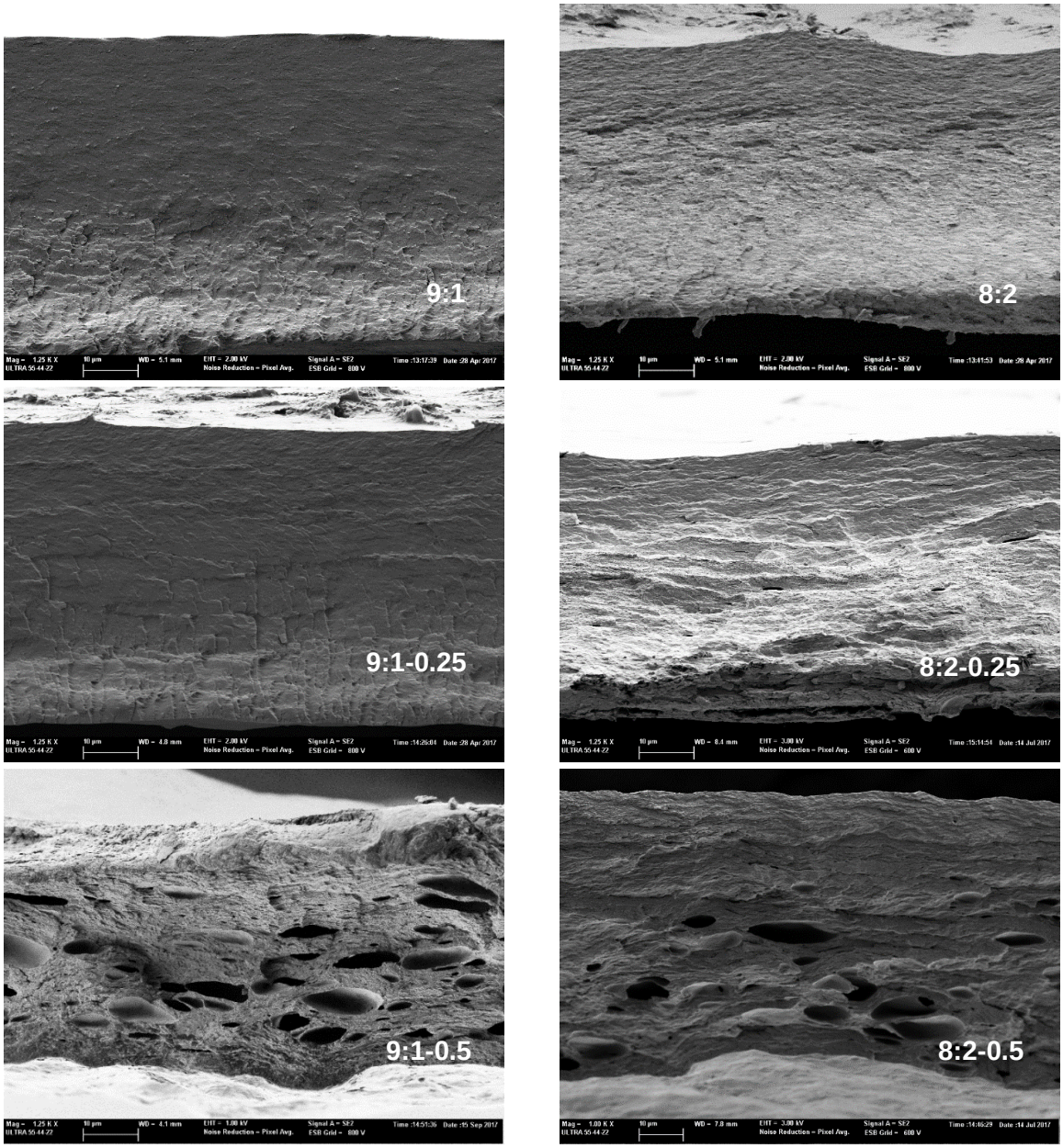

Figure 1. FESEM micrographs of the cross-section of the starch-gellan films with and without EO in the formulations 9:1 (left) and 8:2 (right).

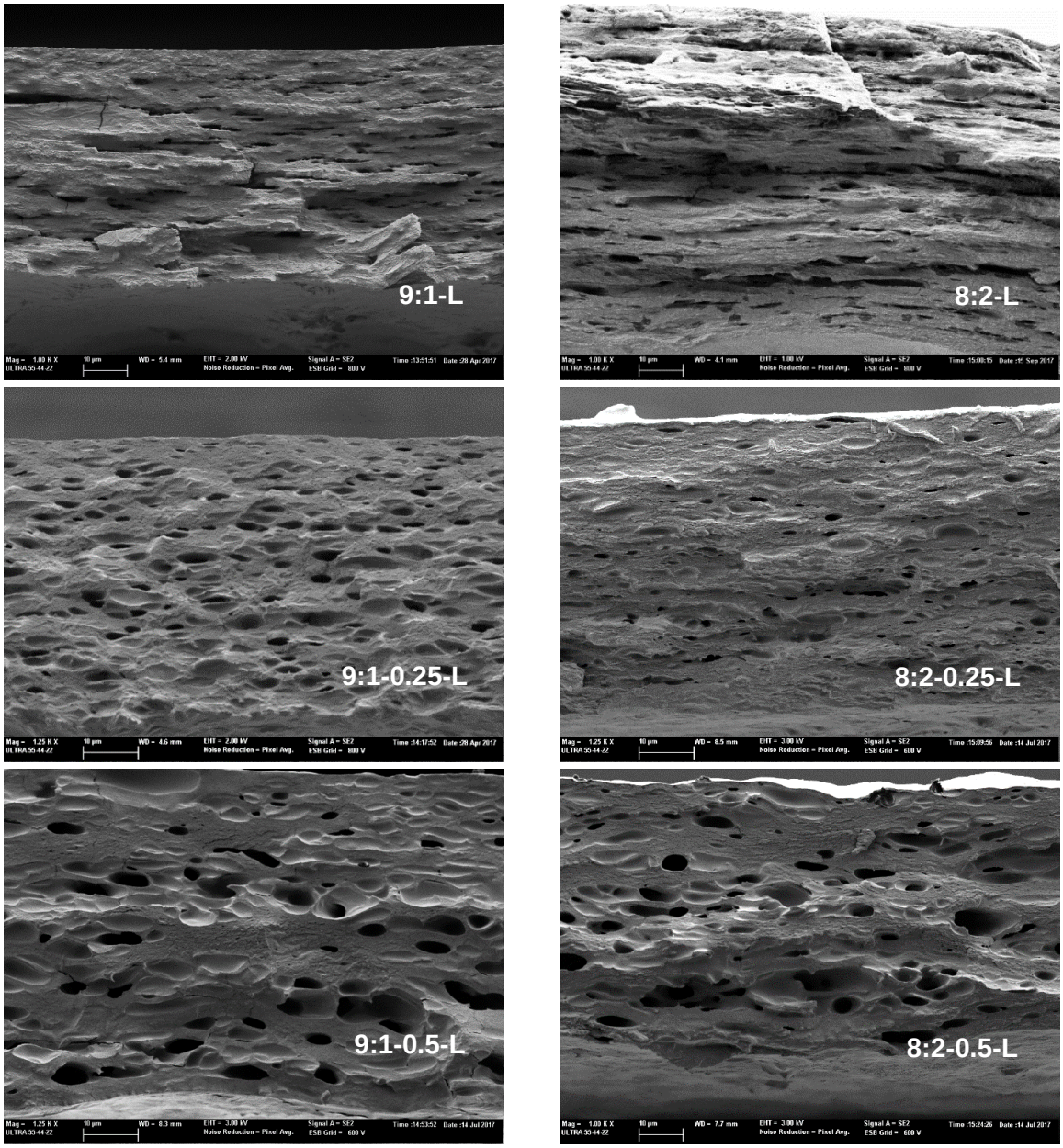

Figure 1. (Continued).

Table 1 shows the theoretical mass fraction of EO added to the film sample, the amount of EO extracted from the different films and the respective retention percentage (with respect to the initial amount) for each sample. A positive aspect of essential oil incorporation as nanoliposomes was the inhibition of oil evaporation during the film drying step, since 42-56 % of the incorporated EO was retained in the dried films. Valencia-Sullca et al. (2016) found similar tendencies in films based on chitosan with the addition of lecithin-encapsulated eugenol and cinnamon leaf essential oil. On the other hand, in films with non-encapsulated EO, the EO retention in the film ranged between 4 and 26 %, and it is notable that the sample with the highest proportion of gellan and non-encapsulated EO retained more oil during the film drying, probably due to the greater viscosity of the dispersion, which helps to stabilize the emulsion,

mainly limiting the creaming of oil droplets to the film surface, where EO quickly evaporates by steam drag effect, in line with water evaporation, as previously reported by (Perdones et al., 2016). Thus, the obtained results show that liposome encapsulation could be a good strategy with which to reduce the EO losses during the film formation process, which coincides with the observations revealed by the microstructural analysis.

Table 1. Nominal mass fraction (X) of the different components in the dried films (P: total polymer, Gly: glycerol, L: lecithin and EO: essential oil) and total amount of EO extracted from dried films, together with the retention percentage (extracted vs. incorporated). Mean values and standard deviation, in brackets.

Sample	X_P	X_{Gly}	X_L	Incorporated EO (g EO/g total solids)	Extracted EO (g EO/g dry film)	% Retained EO in the film
9:1	0.80	0.20	-	-	-	-
8:2	0.80	0.20	-	-	-	-
9:1-L	0.57	0.14	0.29	-	-	-
8:2-L	0.57	0.14	0.29	-	-	-
9:1-0.25	0.67	0.17	-	0.17	0.015 (0.004)^{a}	9 (2)^{a}
8:2-0.25	0.67	0.17	-	0.17	0.006 (0.003)^{a}	4 (2)^{b}
9:1-0.25-L	0.50	0.13	0.25	0.13	0.06 (0.01)^{c}	44 (8)^{cd}
8:2-0.25-L	0.50	0.13	0.25	0.13	0.053 (0003)^{c}	42 (2)^{c}
9:1-0.5	0.57	0.14	-	0.29	0.029 (0.003)^{b}	10 (1)^{a}
8:2-0.5	0.57	0.14	-	0.29	0.074 (0.007)^{d}	26 (2)^{b}
9:1-0.5-L	0.44	0.11	0.22	0.22	0.123 (0.02)^{f}	56 (1)^{e}
8:1-0.5-L	0.44	0.11	0.22	0.22	0.111 (0.015)^{e}	50 (7)^{de}

Different superscript letters within the same column indicate significant differences among films ($p < 0.05$).

3.2. Tensile properties

As concerns tensile behavior, Table 2 shows the values of mechanical parameters (EM, TS and %E) where the effect of the film composition can be observed. All the films with the highest proportion of gellan (8:2), with or without lipids, exhibited greater stiffness (EM) than the corresponding 9:1 S:G films, especially those containing the highest proportion of free EO. Nevertheless, they were less extensible, with the exception of the films with the lowest proportion of free EO, which were the most extensible. Then highest proportion of gellan gave rise to stiffer films with reduced extensibility, probably due to the lack of total miscibility of the polymers, which promoted their brittleness.

Incorporating lipids (EO or L) into the films promoted changes depending on the gellan proportion. The EM decreased in the presence of L or EO, so the films became less stiff, depending on the proportion of G in the matrix. In general, for a given matrix, a greater proportion of lipid (L or EO) led to a greater decrease in both the EM and fracture tension and lower extensibility. However, Valencia-Sullca et al. (2016) found an increase in the extensibility

of chitosan films in the presence of lecithin. The observed differences can be explained by the specific interactions between lipid associations of lecithin with a negative surface charge and positively charged chains of chitosan, which does not occur with the neutral chains of starch and gellan, when the cohesion forces of the polymer network were reduced. Different studies have shown that the incorporation of essential oils usually reduces the mechanical strength of the films as a result of the promotion of a heterogeneous structure with enhanced discontinuities (Jiménez et al., 2014; Jouki et al., 2014). However, small amounts of lipids may plasticize the polymer matrix by reducing the chain interaction forces, without introducing great discontinuities.

3.3. Barrier properties

The WVP, OP and thickness values of the different films are also shown in Table 2. WVP is a relevant property directly related to the usefulness of films in food applications and should be as low as possible to prevent water transfer. The lowest WVP was obtained for films with the lowest ratio of lecithin-encapsulated EO, regardless of the polymer matrix, followed by the other formulations with L (with and without EO). Therefore, the presence of L and the subsequent formation of the layered structure in the film reduced the water vapor transfer rate, mainly due to the resistance offered by the lipid layers in the film. A similar effect was observed by Jiménez et al. (2014), for starch films containing lecithin-encapsulated EO.

However, the incorporation of both L or EO implied an increase in the OP due to the hydrophobic nature of lipids, which facilitates oxygen solubility and transfer (Bertan, Tanada-Palmu, Siani, & Grosso, 2005). This increase was greater in the starch-rich matrix with L, although all the films exhibited very low values of OP, as has been observed for starch-based films (Forssell, Lahtinen, Lahelin, & Myllärinen, 2002).

Table 2. Tensile parameters (elastic modulus, EM; tensile strength, TS; percentage elongation, %E), barrier properties (water vapor permeability, WVP; oxygen permeability, OP) and thickness of the films. Mean values and standard deviation, in brackets.

Sample	EM (MPa)	TS (MPa)	E (%)	WVP ($g\ mm\ KPa^{-1}\ h^{-1}\ m^{-2}$)	OP x 10^{14} ($cm^3\ m^{-1}\ s^{-1}\ Pa^{-1}$)	Thickness (μm)
9:1	1273 (64)^{i}	40 (5)^{g}	4.9 (1.1)^{fg}	6.7 (0.5)^{e}	2.70 (0.04)^{b}	71 (3)^{ab}
8:2	1304 (53)^{i}	24 (5)^{de}	2.1 (0.6)^{ab}	6.2 (0.3)^{d}	2.324 (0.005)^{a}	71 (4)^{ab}
9:1-L	799 (35)^{e}	14 (2)^{bc}	2.0 (0.4)^{ab}	4.2 (0.4)^{b}	6.6 (0.3)^{g}	71 (2)^{ab}
8:2-L	878 (13)^{f}	23 (3)^{d}	3.2 (0.5)^{cd}	4.2 (0.1)^{b}	3.3 (0.2)^{cd}	71 (4)^{ab}
9:1-0.25	745 (86)^{de}	27 (1)^{e}	7.0 (1.0)^{h}	4.8 (0.5)^{c}	3.2 (0.1)^{c}	70 (2)^{a}
8:2-0.25	991 (87)^{g}	35 (5)^{f}	5.5 (1.4)^{g}	4.6 (0.1)^{bc}	3.43 (0.02)^{cde}	70 (3)^{a}
9:1-0.25-L	681 (53)^{cd}	13 (1)^{b}	2.5 (0.4)^{abc}	3.2 (0.2)^{a}	4.5 (0.1)^{f}	73 (2)^{abc}
8:2-0.25-L	707 (80)^{d}	23 (2)^{d}	4.2 (0.5)^{ef}	3.2 (0.3)^{a}	3.5 (0.2)^{cde}	76 (3)^{cd}
9:1-0.5	571 (81)^{ab}	15 (1)^{bc}	3.5 (0.7)^{de}	6.2 (0.8)^{d}	3.8 (0.3)^{e}	74 (6)^{bcd}
8:2-0.5	1072 (34)^{h}	16 (3)^{c}	1.9 (0.2)^{a}	7.1 (0.1)^{e}	3.4 (0.1)^{cd}	73 (6)^{abc}
9:1-0.5-L	514 (74)^{a}	8 (1)^{a}	2.0 (0.5)^{ab}	4.3 (0.2)^{bc}	3.59 (0.01)^{de}	76 (8)^{cd}
8:2-0.5-L	612(32)^{bc}	15 (1)^{bc}	2.7 (0.3)^{bcd}	4.3 (0.1)^{b}	3.3 (0.1)^{cd}	78 (7)^{d}

Different superscript letters within the same column indicate significant differences among films ($p < 0.05$).

3.4. Moisture content and solubility

The values for the moisture content and the water solubility of films are shown in Table 3. As expected, the incorporation of L or EO decreased the equilibrium moisture of the films, although films with free EO exhibited similar or slightly higher values than the lipid-free matrices. As regards water solubility, film formulations with L either with or without EO, exhibited significantly lower solubility values, although free EO did not reduce the water solubility of the films. As reported by some authors (Jouki et al., 2014; Ojagh, Rezaei, Razavi, & Hosseini, 2010), adding EO to the polymer films can promote the film's solubility or water adsorption capacity, which could be attributed to the reduction in the polymer chain interactions in the network, making the water adsorption and film solubilization easier.

3.5. Optical properties

Table 3 also shows the values of the color coordinates (L*, lightness; C_{ab}*, chrome; h_{ab}*, hue) and gloss at 60º of the different films. Due to the typical color of lecithin, films with liposomes were darker, with a more saturated reddish color. Although the lightness was not significantly affected by the incorporation of the active compound, it slightly decreased in the presence of L and EO. In the same way, the hue decreased in the presence of lecithin due to the color contribution of this component. The film gloss increased in matrices with a greater proportion of G, especially after the incorporation of L. Nevertheless, the addition of EO, both in free form or encapsulated, implied a gloss reduction. This can be attributed to an increase in the surface roughness of the film associated with the creaming of the oil drops during the film drying step

and the subsequent oil evaporation, which produces surface irregularities (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009).

Table 3. Water solubility (S, % of soluble solids in the film), equilibrium moisture content (X_w, g/100 g dry film), color coordinates (lightness, chrome and hue) and gloss (60°) of the films. Mean values and standard deviation, in brackets.

Sample	S (%)	X_w	L*	C_{ab}*	h_{ab}*	Gloss (60°)
9:1	38 (9)^{de}	8.2 (0.3)^{d}	81(1)^{c}	4.7 (0.4)^{a}	88.9 (0.4)^{g}	13 (1)^{d}
8:2	48 (2)^{fg}	8.5 (0.1)^{d}	80(1)^{d}	5.4 (0.2)^{b}	87 (1)^{e}	21 (5)^{e}
9:1-L	24 (1)^{abc}	6.2 (0.1)^{a}	76.6 (0.2)^{b}	23.1 (0.5)^{f}	82.7 (0.4)^{c}	52(6)^{g}
8:2-L	31 (2)^{cde}	6.3 (0.1)^{ab}	76.7 (0.4)^{b}	22.9 (0.3)^{f}	81.9 (0.2)^{b}	44 (5)^{f}
9:1-0.25	70 (17)^{h}	8.5 (0.4)^{d}	80.7 (0.4)^{c}	5.4 (0.4)^{b}	88 (1)^{f}	10 (1)^{bc}
8:2-0.25	71 (5)^{h}	8.1 (0.3)^{d}	80.7 (0.5)^{c}	6.3 (0.4)^{c}	86.6 (0.4)^{d}	12 (1)^{cd}
9:1-0.25-L	29 (4)^{bcd}	6.2 (0.1)^{a}	73.0 (0.5)^{a}	21 (1)^{d}	82.6 (0.4)^{c}	13 (2)^{cd}
8:2-0.25-L	20 (1)^{ab}	5.9 (0.3)^{a}	72.2 (0.4)^{a}	21 (1)^{d}	81.8 (0.3)^{b}	7 (1)^{a}
9:1-0.5	57 (2)^{g}	9.7 (1.1)^{e}	76 (8)^{b}	7 (2)^{c}	90 (1)^{h}	9 (1)^{ab}
8:2-0.5	41 (4)^{ef}	9.5 (0.3)^{e}	80.3 (0.3)^{c}	6.9 (0.3)^{c}	87 (1)^{e}	8.0 (0.4)^{ab}
9:1-0.5-L	15 (1)^{a}	7.7 (0.5)^{cd}	73 (1)^{a}	22.3 (0.4)^{e}	79.5 (0.4)^{a}	12 (2)^{cd}
8:2-0.5-L	25 (2)^{abc}	7.1 (0.6)^{bc}	72.4 (0.5)^{a}	22.2 (0.4)^{e}	79.5 (0.3)^{a}	7.2 (0.2)^{a}

Different superscript letters within the same column indicate significant differences among films ($p < 0.05$).

Figure 2 shows the spectral distribution curves of the internal transmittance (T_i) of the films. The incorporation of liposomes, with or without EO, reduced the T_i of the films at low wavelengths due to the brown coloration of L. In contrast, the incorporation of free EO slightly promoted the film transparency of both S:G matrices (9:1 and 8:2), which can be explained by the decrease in the film compactness and, therefore, in the global refractive index. This effect was also observed for lecithin-encapsulated EO.

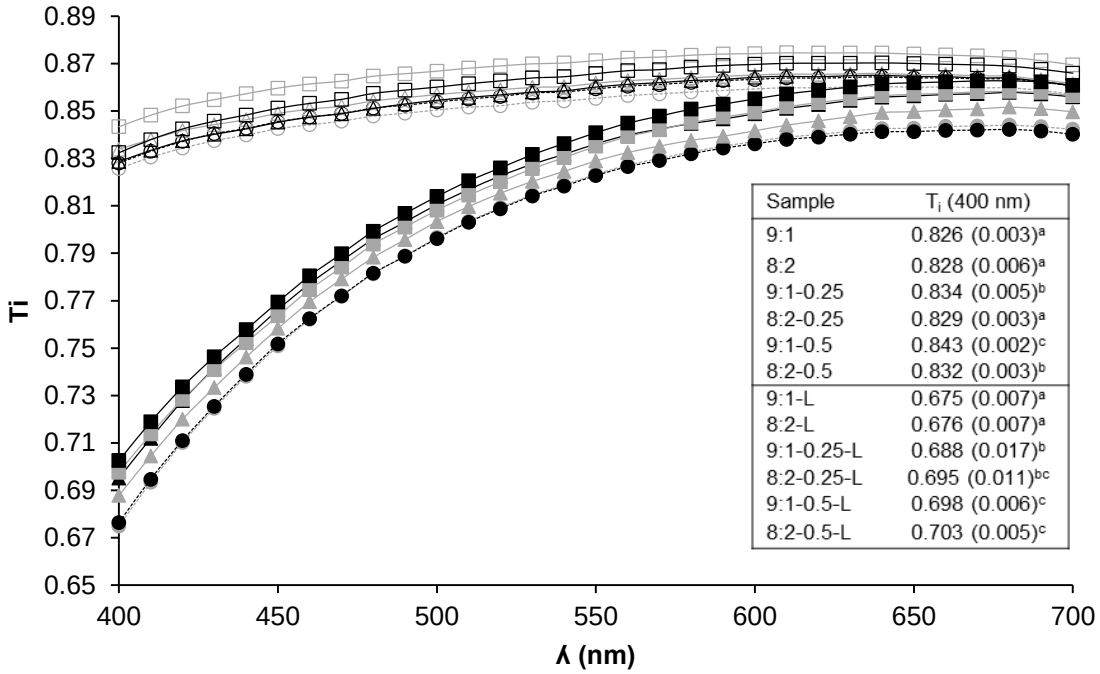

Figure 2. Spectral distribution of internal transmittance (T_i) between 400 and 700 nm for every film formulation with different starch:gellan ratios (grey: 9:1, black: 8:2) containing EO (0.25 or 0.5 g/g polymer: triangle and square, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols), and without EO (control: circles). Embedded table shows the T_i values at 400 nm and the ANOVA carried out separately for samples with and without lecithin. Different superscript letters within the same column indicate significant differences among films ($p < 0.05$).

3.6. Antifungal properties

The antifungal effect exhibited by thyme EO varied depending on the formulation and residual content of EO in the films. Fig. 3 shows the radial growth of each fungus (AA and BC) for the control films (without EO) and the films containing EO applied on the culture plate. A linear fungus growth was observed as a function of time for every sample, and the fitted straight lines ($r^2 > 0.872$) were obtained, with the corresponding slope (growth rate) and intercept, related to the total growth inhibition time (t_0) for each fungus. Table 4 shows the obtained values of GR (slope) and the intercept of the fitted straight lines in each case, as well as the estimated value of the total inhibition period (t_0) and the mycelial growth inhibition (MGI) at 7 days. In terms of its antifungal action, it is remarkable that thyme EO was more effective against *B.cinerea* than *A. alternata*. In fact, for an EO concentration in the films of 0.074 g/g dried film (16.6 mg per plate or 0.8 mg/mL of medium), no growth of *B. cinerea* was observed throughout the tested period, thus indicating a total fungicide action. Likewise, GR values were lower for *B. cinerea* than for *A. alternata* for a determined EO content in the film. It is remarkable that no notable differences in GR were observed for *A. alternata* fungus for EO contents in the film between 0

and 6-7% (Figure 4), whereas a sharp decrease in GR was observed for higher contents. In general GR values were slightly lower for the 8:2 S:G matrices for a determined EO content. For *B. cinerea* a linear decrease of GR as a function of the EO content in the 8:2 S:G matrices was observed, whereas it fluctuated between 2.5-1.1 for the 9:1 S:G matrices depending on the EO content or presence of L. In contrast, t_0 values rose when the EO content in the films increased. This behavior suggests that the fungal growth was inhibited for a determined time (t_0) when films contained EO in different proportion, but the EO volatilization or the adaptation of the fungi allows for subsequent growth at the same rate as in the control samples (coated with EO-free films). Only above a critical EO concentration, was the development of the fungal affected (lower GR), indicating cellular alterations affecting their vital activity. This was clearly observed in *A. alternata* and less noticeable in *B. cinerea*, which in turn exhibited greater sensitivity to the active EO.

In *A. alternata*, an effect of the film's matrix composition (9:1 or 8:2 S:G ratio) was observed both in the control samples and active films. The higher content of starch enhanced fungal growth, limiting the action of EO in the cases where films contained a low content of the active. At a higher EO content, the effect of the matrix was less remarkable. This could be clearly seen when the GR and t_0 values were correlated with the real content of EO in the films (Figure 4). In particular, two different relationships could be observed between the t_0 values and the EO content in the film for the two matrices. In 9:1 S:G matrices, a good linear correlation of t_0 and EC content was observed, whereas more fluctuating, generally higher values were observed for 8:2 S:G films. This also points to the nutritional effect of film starch on the fungi, which enhanced their vitality and defense against the antifungal compounds. GR fluctuated between 4-5 mm day^{-1} when the EO content was lower than 6-7 % in the dried film and decreased sharply to 1-2 mm day^{-1} for higher contents of the EO. This suggests that the films require relatively high contents of EO to ensure a good antifungal action against *A. alternata*. These concentrations were only reached when lecithin encapsulation was used to prevent losses of the EO during the film drying step or when the S:G ratio was 8:2 in the matrix and EO was incorporated at the highest ratio (0.5 with respect to the polymer in the initial dispersion). The best fungal control was achieved with the 9:1 (S:G) film formulation, with lecithin-encapsulated EO (at a nominal ratio of 0.5), which retained the highest EO content in the film matrix.

In the case of *B. cinerea*, all the films with EO content ≥ 0.074 g/g dried film completely inhibited the growth of the fungus. At lower levels of EO, similar tendencies to those commented on above for *A. alternata* were observed, although the nutritional effect of starch on the fungus was scarcely noticeable when films contained EO, probably due to the greater sensitivity of this fungus to the antifungal action of the EO. A linear relationship could be observed for t_0 vs. EO content for all of the active films, the slope being higher than that obtained for *A. alternata*, in line with the greater fungus sensitivity, with small differences between the matrices with different starch ratios or the presence of lecithin. Likewise, the GR fluctuated from 0.7 to 2.5 mm day^{-1} as a function of the EO content in the film and the starch ratio in the matrix. The higher the EO content and the lower the starch ratio, the lower the GR values. Of the film formulations allowing fungal growth, the one containing 0.25 lecithin-encapsulated EO in an

8:2 S:G matrix was the most effective at controlling the fungal development, despite this film retaining a slightly lower amount of the EO than the corresponding 9:1 S:G film, thus reflecting the role starch plays in the protection of the fungus's vitality.

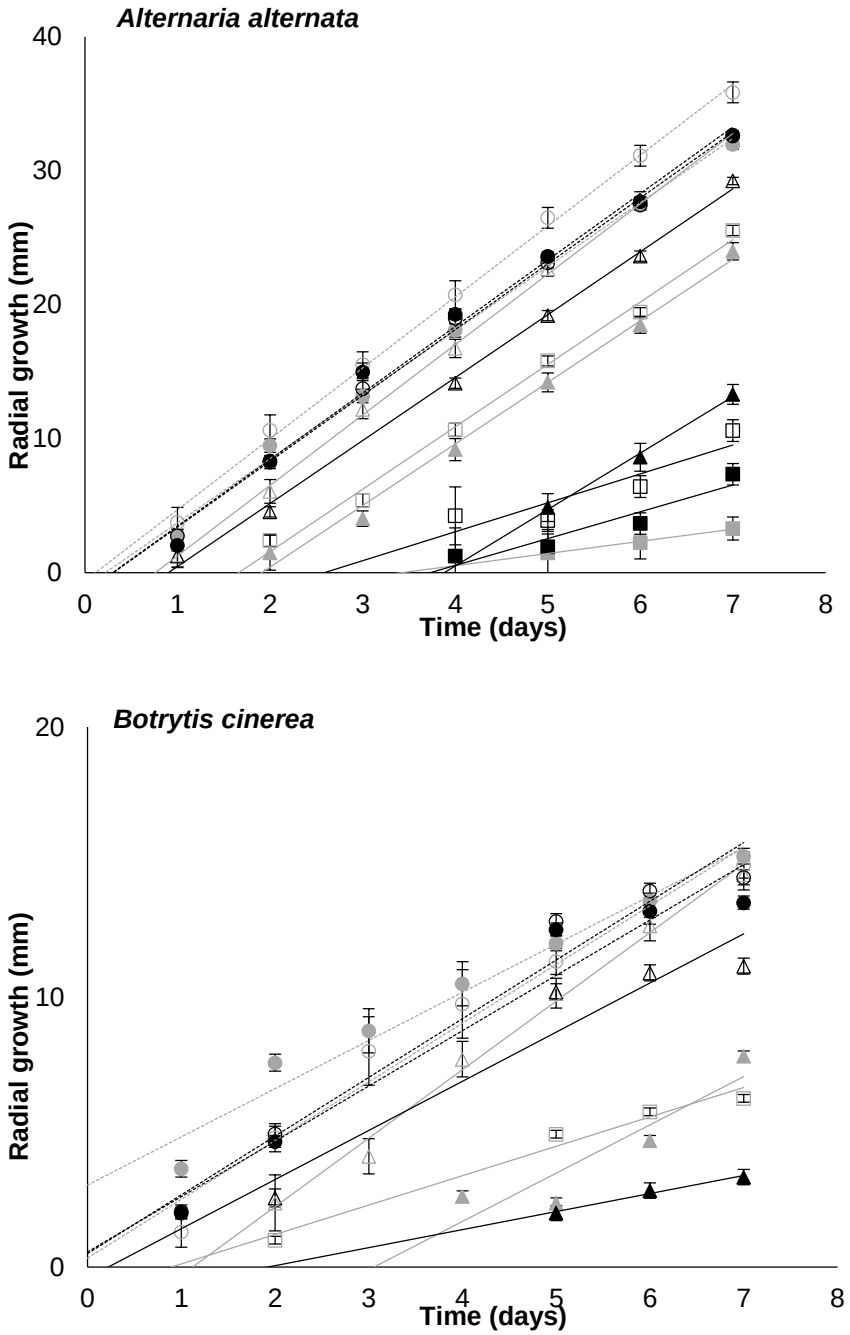

Figure 3. Radial growth of *Alternaria alternate* and *Botrytis cinerea* for every film formulation with different starch:gellan ratios (grey: 9:1, black: 8:2) containing EO (0.25 or 0.5 g EO/g polymer: triangles and squares, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols) and without EO (control: circles).

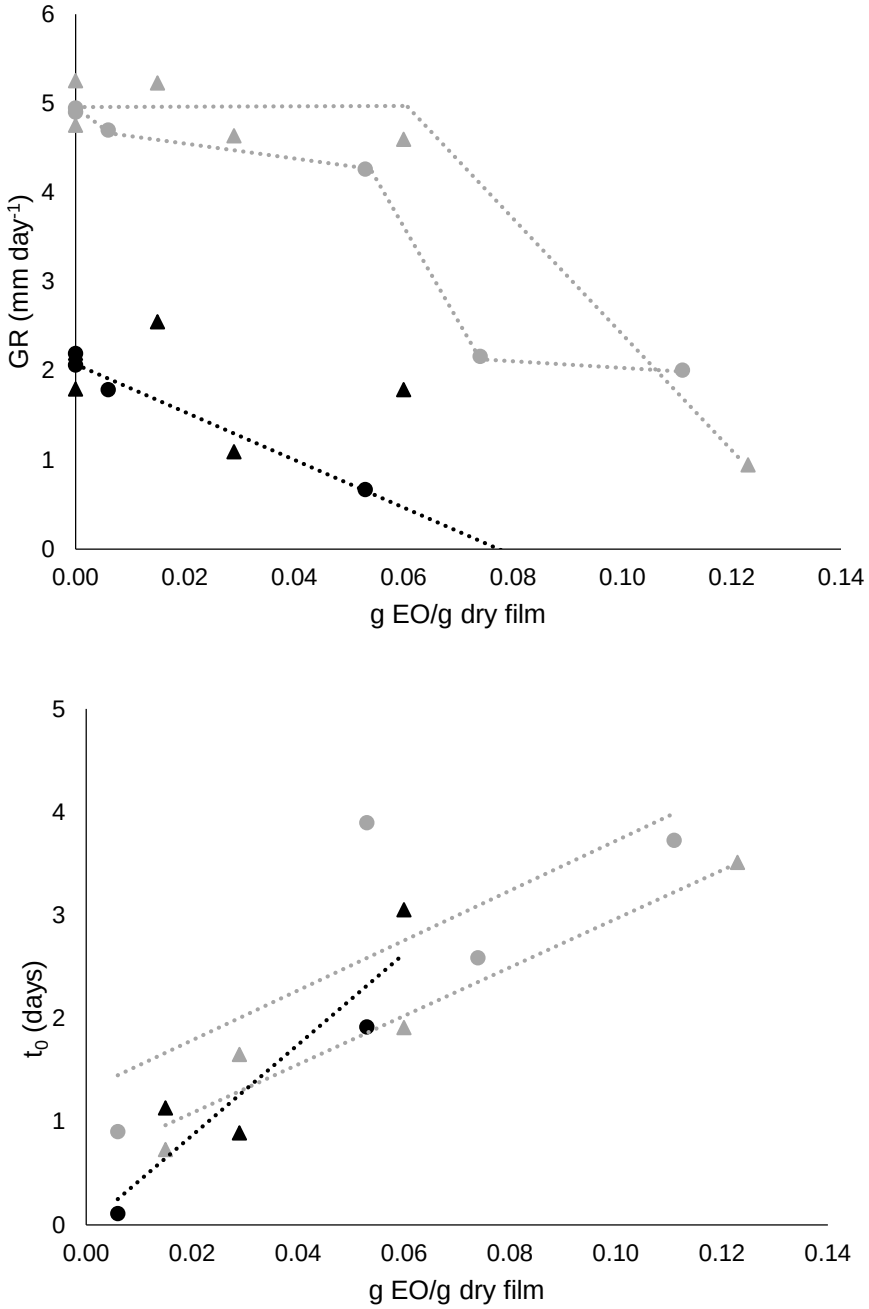

Figure 4. Relationships between GR and t_0 with the actual EO concentration in the films with different S:G ratios (9:1:, triangles, 8:2: circles) for both fungi (AA: grey and BC: black).

Table 4. Slope (growth rate: GR, mm day^{-1}), intercept of the straight lines and estimated value of the total inhibition period (t_0, days) for *Alternaria alternata* and *Botrytis cinerea*. Mycelial growth inhibition (MGI, % values) at 7 days was also included.

	Alternaria alternata				*Botrytis cinerea*			
Sample	GR (slope)	Intercept	t_0	MGI	GR (slope)	Intercept	t_0	MGI
9:1 (*Control)*	5.3 (0.2)	-0.4 (0.9)	0.07	-	2.2 (0.1)	0.3 (0.5)	-	
9:1-0.25	5.2 (0.1)	-3.8 (0.7)	0.73	9.9	2.5 (0.2)	-2.9 (0.9)	1.13	2.4
9:1-0.5	4.6 (0.1)	-7.6 (0.6)	1.65	28.8	1.1 (0.1)	-1.0 (0.3)	0.90	58.0
9:1-L (*Control)*	4.8 (0.1)	-0.9 (0.4)	0.19	-	1.8 (0.1)	2.9 (0.3)	-	
9:1-0.25-L	4.6 (0.2)	-8.8 (0.9)	1.91	24.9	1.8 (0.2)	-5.5 (0.9)	3.05	48.7
9:1-0.5-L	0.9 (0.9)	-3.3 (5.6)	3.51	89.6	-	-	-	100
8:2 (*Control)*	4.9 (0.1)	-1.5 (0.4)	0.31	-	2.2 (0.1)	0.4 (0.4)	-	
8:2-0.25	4.7 (0.1)	-4.2 (0.4)	0.90	10.5	1.8 (0.1)	-0.2 (0.7)	0.11	22.8
8:2-0.5	2.2 (0.5)	-5.6 (3.1)	2.59	67.5	-	-	-	100
8:2-L (*Control)*	4.9 (0.1)	-1.6 (0.4)	0.32	-	2.1 (0.1)	0.5 (0.4)	-	
8:2-0.25-L	4.3 (0.6)	-16.6 (3.9)	3.90	59.2	0.7 (0.2)	-1.3 (1.2)	1.92	75.3
8:2-0.5-L	2.0 (0.5)	-7.5 (3.2)	3.72	77.4	-	-		100

4. CONCLUSIONS

Starch-gellan blend films containing thyme essential oil (EO) exhibited antifungal effect in *in vitro* tests against *Alternaria alternata* and *Botrytis cinerea,* the second being more sensitive to the action of the EO. The antifungal action was correlated with the residual content of the oil in the film after the drying step and was slightly affected by the polymer matrix composition (9:1 or 8:2 S:G ratio). A greater amount of starch in the film protected the fungi, making their growth faster, when the active content was relatively low. The growth of AA was greatly inhibited when the EO content exceeded 0.05 g/g film, whereas BC was completely inhibited when films contained more than 0.053 g EO/ g film. Lecithin encapsulation of the EO greatly contributed to the EO retention in the film during film formation, which enhanced the film's antifungal action. Therefore, lecithin enhanced the film's water barrier properties, whereas all of the films exhibited high oxygen barrier capacity. Lecithin imparted a slightly brownish color to the films, but improved their gloss while reducing film stiffness and resistance to break and extensibility. Then, films with lecithin-encapsulated EO, with a S:G ratio of 8:2 were very effective at controlling fungal growth, while exhibiting adequate functional properties as packaging/coating materials.

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CHAPTER 3. Wettability of starch-gellan coatings on fruits, as affected by the incorporation of essential oil and/or surfactants

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ABSTRACT

Wettability of coating-forming systems (CFS) based on starch-gellan (80:20) blends, containing, or not, emulsified/lecithin-encapsulated thyme essential oil (EO), was analysed in apple, tomato and persimmon fruits. Different concentrations of Tween 85 were incorporated into the CFS in order to know its potentially beneficial effect on the coating spreadability. These fruit skins exhibited high values of the surface tension dispersive component, while being low-energy surfaces. Values of contact angle and surface tension of the starch-gellan solutions were positively affected by the addition of Tween 85 at $5 \cdot 10^4$ mg/L. However, it exerted a negative effect when CFS contained emulsified or lecithin-encapsulated thyme essential oil. Likewise, wettability of starch-gellan coatings was notably improved with Tween 85 at $5 \cdot 10^4$ mg/L, whereas formulations containing emulsified or encapsulated EO did not require surfactant to improve their already good spreadability.

Keywords: cassava starch; gellan; edible coating; surface properties; wettability.

1. INTRODUCTION

One of the most important problems in fruit trading is their short shelf life, which produces heavy losses from harvesting to final consumption (Cerqueira, Lima, Teixeira, Moreira, & Vicente, 2009; Flores-López, Cerqueira, de Rodríguez, & Vicente, 2016). Their shelf-life can be prolonged by reducing fruit respiration or gas transfer rates through the control of factors, such as the composition of the atmosphere surrounding the fruit (O_2, CO_2 and ethylene), temperature, water vapour transfer rate or relative humidity (Lima et al., 2010).

The application of edible coatings, using biopolymers, as a postharvest technique for agricultural commodities, offers environmentally-friendly alternatives in order to solve these problems. Several studies have demonstrated that the application of polysaccharide-based coatings on fruits and vegetables, such as apple (Carneiro-da-Cunha et al., 2009; Mehyar, Al-Qadiri, & Swanson, 2014), strawberry (Garcia, Pereira, de Luca Sarantópoulos, & Hubinger, 2010; García, Martino, & Zaritzky, 1998; Ribeiro, Vicente, Teixeira, & Miranda, 2007), orange (Saberi et al., 2018), guava (Botelho, Rocha, Braga, Silva, & de Abreu, 2016; De Aquino, Blank, & De Aquino Santana, 2015) or tomato (Nawab, Alam, & Hasnain, 2017; Ortega-Toro, Collazo-Bigliardi, Roselló, Santamarina, & Chiralt, 2017), provides an extended shelf life while enhancing the product quality.

Starch is a promising polysaccharide for food coating/packaging purposes, when considering its filmogenic capacity, ready availability, renewability and low cost (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015). Starch-based coatings are colourless, odourless, have an oil-free appearance and exhibit low oxygen permeability which can contribute to reducing the respiration rate of the fresh products. It can be used in combination with other biopolymers in order to overcome some drawbacks, such as high water sensitity and water vapour permeability or the retrogradation phenomena during storage (Cano, Jiménez, Cháfer, Gónzalez, & Chiralt, 2014). In this sense, different microbial gums, such as gellan or xanthan gums, have been described as enhancers of the mechanical resistance of starch films, while limiting their water sensitivity and retrogradation (Arismendi et al., 2013; Kim, Choi, Kim, & Lim, 2014; Sapper, Talens, & Chiralt, 2019). Gellan gum at 10 and 20 % in starch blends improved the functional properties of the starch matrix, while also carrying active compounds (thyme essential oil) to better control the product shelf-life through their antifungal effect (Sapper, Wilcaso, Santamarina, Roselló, & Chiralt, 2018).

An important issue related with the effectiveness of edible coatings at preserving fruits and vegetables is the product surface wettability with the coating which determines the coating uniformity and thickness on the surface (Cerqueira et al., 2009; Park, 1999) and, thereby, its permeability and mechanical performance. The coating process involves the wetting of the surface to be coated, the possible penetration of the coating solution into the peel, followed by the potential adhesion between the coating solution and the food surface (Hershko, Klein, & Nussinovitch, 1996). In this sense, the fruit surface energy and liquid surface tension are controlling factors, affecting the contact angle and liquid spreadability on the solid surface (Hong, Han, & Krochta, 2004). Then, this analysis, which includes the estimation of the critical

surface tension of the solid surface, is relevant to evaluate the effectiveness of the product wetting.

The wetting behaviour is influenced both by the chemical composition (e.g., polymer, plasticisers, surfactants, antimicrobials or antioxidants) and molecular interactions in the coating-forming solutions, and by the product surface interactions with the coating components (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). Consequently, it is important to optimise the coating formulations in order to promote their spreading coefficient (W_s) on a determined surface. This coefficient is a function of the work of cohesion (W_c) in the liquid phase and the work of adhesion (W_a) between liquid and solid surface (Osorio et al., 2017). The effect of the composition of coating solutions on the wetting properties of different fruits and vegetables has been described by several authors. In this sense, Carneiro-da-Cunha et al. (2009) and Choi, Park, Ahn, Lee, & Lee (2002) reported that the addition of Tween 80 was effective in reducing the surface tension of different coating solutions, which improved the compatibility between the solution and the surface of the fruit skin. Otherwise, different formulations of galactomannans and glycerol coatings showed good values of W_s when applied on different tropical fruits (Cerqueira et al., 2009). Likewise, the addition of glycerol and cellulose nanofibres enhanced the wetting of banana and eggplant with coating formulations based on gelatin (Andrade et al., 2014).

The aim of this study was to characterise the surface properties of apple, tomato and persimmon and to evaluate the wettability/spreading coefficient of the starch-gellan coating-forming liquids, containing, or not, free or lecithin-encapsulated thyme essential oil, as affected by the addition of different concentrations of Tween 85.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Cassava starch (S) (Quimidroga S.A., Barcelona, Spain), low acyl gellan gum (G) (KELCOGEL F, Premium Ingredients, Murcia, Spain), non-GMO soy lecithin with 45% phosphatidylcholine (L) (Lipoid P45, Lipoid GmbH, Ludwigshafen, Germany), thyme (*Thymus zygis*) essential oil (EO) (Plantis, Artesanía Agrícola SA, Barcelona, Spain), glycerol (Panreac Química S.A., Barcelona, Spain) and polyoxyethylenesorbitan trioleate (Tween 85) (T) (Sigma-Aldrich, Madrid, Spain), were used to obtain the coating-forming systems (CFS). Heptane (Sigma.Aldrich, Madrid, Spain), dimethyl sulfoxide (DMSO) and methanol (Panreac Química S.A., Barcelona, Spain) were used as reference compounds.

2.2. Preparation of the coating-forming systems (CFS)

To obtain the CFS, S (2 % w/w) was dispersed in distilled water and heated to 95 ºC for 30 min, while hand stirring, to induce complete starch gelatinization. The G solution (2 % w/w) was obtained under magnetic stirring at 90 ºC for 60 min. Both were cooled down to reach room temperature and afterwards, glycerol was incorporated as plasticizer (0.25 g/g of polymer), on the basis of previous studies (A. Cano et al., 2015; Jiménez, Fabra, Talens, & Chiralt, 2012). The S and G solutions were mixed in 8:2 ratio to obtain the solutions without EO (S:G formulations). The EO, used as an antifungal compound (0.5 g/g of polymer), was added either by direct emulsification or encapsulated in lecithin liposomes (ratio polymer: lecithin 1: 0.5). In the first case (S:G-EO formulations), the EO was incorporated directly and the dispersions were homogenized for 3 min at 13500 rpm using a rotor-stator homogenizer (Ultraturrax Yellow Line DL 25 Basic, IKA, Staufen, Germany). In the second case (S:G-EO-L formulations), the liposome dispersions were previously prepared and added directly to the polymer blend solution while kept under soft magnetic stirring for 2 h. Lecithin dispersions (LD) were prepared following the method described by Valencia-Sullca et al. (2016). Lecithin (5 % w/w) was dispersed in water and stirred for at least 4 h at 700 rpm. The EO (5 % w/w) was incorporated into the lecithin dispersion by using a sonicator (Vibra Cell, Sonics & Materials, Inc. USA) at 20 kHz for 10 min with pulses of 1 s. Tween 85 was added to the different formulations at different concentrations from 0 to 10^5 mg/L of total solution. All of the solutions were degassed by using a vacuum pump (MZ 2C NT, Vacuubrand GmbH + CO KG, Germany).

2.3. Surface properties of the fruits

Apples (*Malus domestica* Borkh cv. Golden Delicious), tomatoes (*Lycopersicom esculentum*) and persimmons (*Diospyros kaki* Thunb. Cv. Rojo Brillante) were purchased from a local market (Valencia, Spain). Fruits of uniform size, shape and colour and without any signs of mechanical damage were selected, cleaned with 1 % sodium hypochlorite solution and dried

at room temperature. To determine the contact angles, thin sections of the skin were cut and placed on a glass plate to proceed with the measurements.

2.3.1. Contact angle and surface tension measurements

The contact angle (θ) and liquid-vapour surface tension (γ_L) were measured by means of a Dynamic Contact Angle measuring devices and Tensiometer (OCA 20, DataPhysics Instruments GmbH, Filderstadt, Germany). The contact angles were measured by the sessile drop method (Kwok & Neumann, 1999), in which a droplet of the tested liquid was placed on the horizontal surface with a needle of 1.19 mm of internal diameter. Measurements were made in less than 10 s. The surface tension of the CFS was measured by the pendant drop method. Image analyses were carried out using SCA20 software. At least twelve replicates were obtained for each parameter and formulation.

2.3.2. Surface tension and critical surface tension of fruits skins

Surface tension of the fruit skins were determined through the measurement of contact angles (θ) on the fruit surface (Eq. (1)) of at least three different polar and nonpolar liquids: distilled water, dimethyl sulfoxide, methanol and heptane. Their surface tension (γ_L) parameters (values of polar (γ_L^p) and dispersive (γ_L^d) components) are shown in Table 1. According to Eq. (2) which describes the work of liquid-solid adhesion (W_a), the Eq. (3) can be obtained. From Eq. (3), the polar (γ_S^p) and dispersive (γ_S^d) contributions of the solid surface tension can be obtained by plotting the values of the dependent variable $\left(\frac{1+\cos\theta}{2}\frac{\gamma_L}{\sqrt{\gamma_L^d}}\right)$ vs. the independent variable $\left(\sqrt{\frac{\gamma_L^p}{\gamma_L^d}}\right)$, both calculated from the experimental values of θ of the different liquids with known values of γ_L, γ_L^p and γ_L^d.

$$cos\,\theta = \frac{\gamma_S - \gamma_{SL}}{\gamma_L} \tag{1}$$

$$W_a = W_a^d + W_a^p \leftrightarrow W_a = 2\cdot\left(\sqrt{\gamma_S^d\cdot\gamma_L^d} + \sqrt{\gamma_S^p\cdot\gamma_L^p}\right) = \gamma_L(1+\cos\theta) \tag{2}$$

$$\frac{1+\cos\theta}{2}\cdot\frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_S^p}\cdot\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_S^d} \tag{3}$$

2.3.3. Wettabilitty

The wettability of coatings on the fruit surface was determined as the spreading coefficient (W_s), depending on of the works of adhesion (W_a) (Eq. (4)) and cohesion (W_c) (Eq. (5)), in terms of surface tensions at the interfaces *SV* (γ_S), *LV* (γ_L) and *SL* (γ_{SL}) and contact angle (Eq.(6)) (Rulon & Robert, 1993).

$$W_a = \gamma_L + \gamma_S - \gamma_{SL} = \gamma_L(1 + \cos\theta) \quad (4)$$

$$W_c = 2 \cdot \gamma_L \quad (5)$$

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{SL} = \gamma_{LV}(\cos\theta - 1) \quad (6)$$

2.4. Statistical analysis

Statistical analyses were performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVI.II (StatPoint Technologies Inc., Warrenton, VA, USA). Fisher's least significant difference (LSD) procedure was used at the 95 % confidence level.

3. RESULTS AND DISCUSSION

3.1 Fruit surface properties

The estimation of the critical surface tension (γ_C) of the different fruit skins was carried out by extrapolation from a Zisman plot (Zisman, 1964) (Figure 1). For a simple molecular liquid, γ_C is essentially independent of the nature of the liquid and only depends on the food surface (Andrade et al., 2014). The cosine of the contact angle obtained for the pure liquids on the fruit surface (Table 2) was plotted *vs*. the respective surface tension of the liquids, γ_L (Table 1). From the fitted straight lines, the intercept with cos θ = 1 corresponds to the γ_C values of each surface (Eq. (7)). This corresponds to the value of the surface tension (liquid/vapour), which would promote the best surface wettability. This method is applicable only for systems with a surface tension below 100 mN/m (low-energy surfaces). Thus, it is important to determine the surface energy of the target solid in order to verify its applicability.

$$\gamma_C = \lim_{\gamma LV} \text{ as } \theta \rightarrow 0 \quad (7)$$

Table 3 shows the values of the critical surface tension of apple, tomato and persimmon surfaces. In general, the critical surface tension values are lower than the solid surface tension (Dann, 1970). This was verified in this study, as also reported by different authors for mango and apple skins (Lima et al., 2010), tomato and carrot (Casariego et al., 2008) and strawberry (Ribeiro et al., 2007).

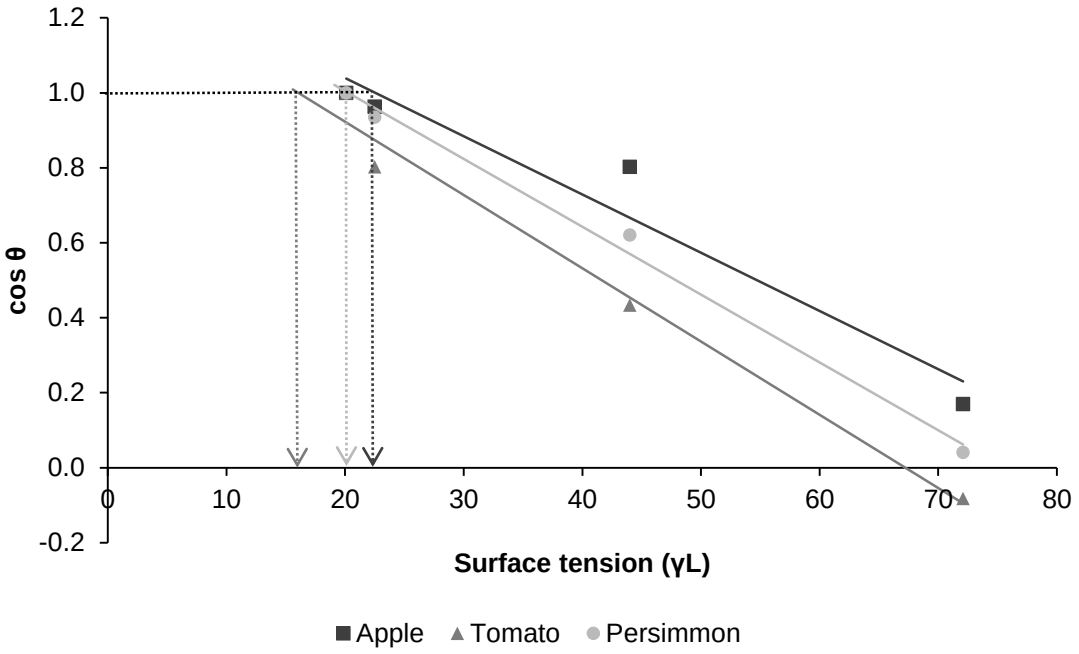

Figure 1. Zisman plot for apple, tomato and persimmon surfaces.

Table 1. Surface tension components of the reference liquids used for characterisation of the fruit surfaces (mN/m): surface tension (γ_L), dispersive (γ_L^d) and polar (γ_L^p) components.

Component	Compound			
	Water[a]	Methanol[b]	DMSO[b]	Heptane[b]
γ_L	72.1	22,5	44.0	20.1
γ_L^d	19.9	18.2	36.0	20.1
γ_L^p	52.2	4.3	8.0	0.0

[a] Data from Busscher, van Pelt, de Boer, de Jong, & Arends (1984).

[b] Data from Accu Dyne Test (2008).

Table 2 shows the values of contact angles of the different pure liquids on the surface of apple, tomato and persimmon fruits. From these values and the surface tension components of the liquids (Table 1), the independent $\left(\frac{1+\cos\theta}{2}\cdot\frac{\gamma_L}{\sqrt{\gamma_L^d}}\right)$ and dependent $\left(\sqrt{\frac{\gamma_L^p}{\gamma_L^d}}\right)$ variables of Eq. (2) were estimated. Eqs. (8) to (10) were fitted (Figure 2) to the respective data obtained for apple, tomato and persimmon surfaces.

Table 2. Contact angle (θ) values measured with the different pure liquids on the skin of apple, tomato and persimmon. Mean values and standard deviations.

Surface	Compound			
	Water	Methanol	DMSO	Heptane
Apple	80 ± 3	16 ± 5	37 ± 5	0
Tomato	95 ± 2	37 ± 4	64 ± 3	0
Persimmon	88 ± 5	21 ± 3	52 ±3	0

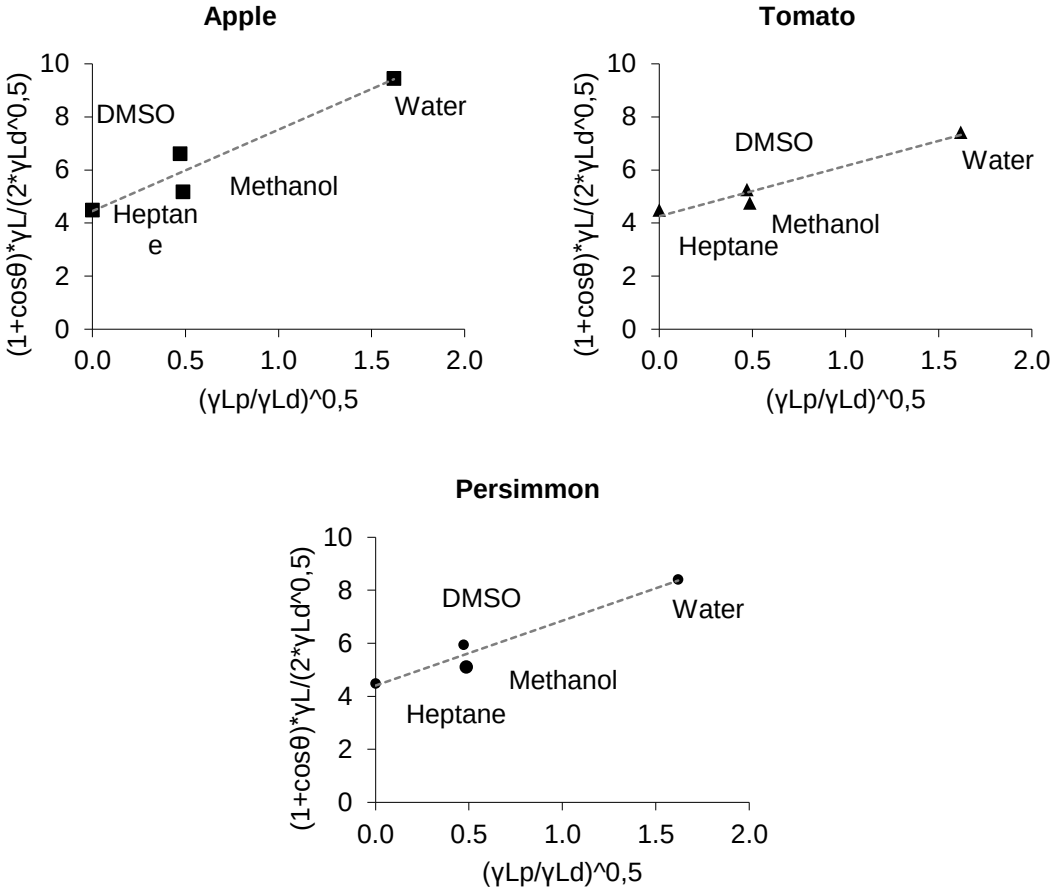

Figure 2. Polar and dispersive components of apple, tomato and persimmon surfaces.

$$\frac{1+\cos\theta}{2}\frac{\gamma_L}{\sqrt{\gamma_L^d}} = 3.1\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + 4.5;\ r^2 = 0.9246 \quad (8)$$

$$\frac{1+\cos\theta}{2}\frac{\gamma_L}{\sqrt{\gamma_L^d}} = 1.9\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + 4.3;\ r^2 = 0.9526 \quad (9)$$

$$\frac{1+\cos\theta}{2}\frac{\gamma_L}{\sqrt{\gamma_L^d}} = 2.4\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + 4.4;\ r^2 = 0.9554 \quad (10)$$

The slope and intercept of the fitted equations were used to estimate the values of the polar and dispersive components, respectively, as well as the total surface tension of the fruit surfaces. These values, also shown in Table 3, clearly demonstrate that apple, tomato and persimmon have low-energy surfaces (lower than 100 mN/m), and that their surface interactions with liquids would mainly be given by dispersion forces. This is coherent with the

natural waxy coatings of the fruit, where non-polar components are the main constituents. Due to the high values of the dispersive component of the surface tension, these surfaces would have the ability to participate in non-polar interactions (Cerqueira et al., 2009). The tomato surface was the one that showed the lowest value of the polar component, and therefore, the most limited ability to participate in polar interactions with hydrophilic coatings. Values obtained for apple and tomato were in the range of those reported by other authors (Carneiro-da-Cunha et al., 2009; Casariego et al., 2008; Lima et al., 2010), although no previous studies were reported for persimmon.

Table 3. Values of the surface properties determined for the skin of apple, tomato and persimmon (mN/m).

Fruit	Polar component (γ_S^p)	Dispersive component (γ_S^d)	Solid surface tension	Critical surface tension (γ_c)
Apple	9.4	19.8	29.2	22.7
Tomato	3.6	18.2	21.7	16.1
Persimmon	6.0	19.4	25.4	20.3

3.2. Fruit wettability

Values of the contact angles of the three formulations with the different amounts of Tween 85 are shown in Table 4. As concerns the formulations without Tween 85, the contact angle decreased when they contained EO, either emulsified or lecithin-encapsulated. This suggests that emulsified EO and liposomes affected the interactions of the CFS with the fruit/air interfaces. The lowest value was reached in all cases for persimmon, while more similar values were obtained for apple and tomato for every formulation. As expected, the incorporation of Tween 85 modified the values of contact angles, depending on the concentration and coating composition. In general, the contact angle decreased as the concentration of Tween 85 rose, but the most significant change occurred at a high concentration level (about 10^4 mg/L). This suggests that interactions of the surfactant molecules within the system hindered the surface activity of the compound at low concentrations, which can limit their effectiveness at favouring the coating spreading on the fruit surface. In this sense, several authors (Eliasson, 1994; Ghiasi, Varriano-Marston, & Hoseney, 1982; Mira, Eliasson, & Persson, 2005; Wokadala, Ray, & Emmambux, 2012) have described the complexes of amylose with surfactants, which can give rise to low amounts of free surfactant molecules to act at surface level. On the other hand, when the EO is present in the system, the surfactant molecules could be predominantly adsorbed on the oil droplet surface, thus being less available for acting at the solid-liquid-vapour interfaces. For systems with lecithin-encapsulated EO, the surfactant molecules could interact with the lecithin membranes, which could also compromise the ability of the molecules to promote the liquid spreading on the fruit surface. The highest contact angle was obtained on apple skin with the starch-gellan dispersion without Tween 85.

Table 4. Contact angle (θ) values of the different CFSs on the skin of apple, tomato and persimmon as a function of the Tween concentration (mg/L). Mean values and standard deviations.

Sample	Fruit	0	10^0	10^1	10^2	10^3	10^4	$5{\cdot}10^4$	10^5
S:G	Apple	$96 \pm 2^{g,3}$	$95 \pm 3^{fg,3}$	$94 \pm 2^{ef,2}$	$93 \pm 3^{e,2}$	$91 \pm 2^{d,2}$	$71 \pm 4^{c,3}$	$44 \pm 2^{a,2}$	$47 \pm 3^{b,2}$
	Tomato	$92 \pm 2^{de,2}$	$92 \pm 1^{de,2}$	$92 \pm 1^{e,2}$	$91 \pm 2^{d,2}$	$91 \pm 2^{de,2}$	$67 \pm 4^{c,2}$	$48 \pm 2^{a,3}$	$50 \pm 2^{b,3}$
	Persimmon	$67 \pm 3^{d,1}$	$68 \pm 4^{d,1}$	$75 \pm 5^{e,1}$	$77 \pm 6^{e,1}$	$86 \pm 3^{f,1}$	$52 \pm 4^{c,1}$	$42 \pm 3^{a,1}$	$46 \pm 2^{b,1}$
S:G-EO	Apple	$77 \pm 2^{f,3}$	$69 \pm 2^{e,3}$	$70 \pm 1^{e,2}$	$70 \pm 2^{e,2}$	$64 \pm 2^{d,2}$	$43 \pm 3^{a,2}$	$44 \pm 2^{b,2}$	$57 \pm 2^{c,3}$
	Tomato	$73 \pm 2^{e,2}$	$71 \pm 2^{d,2}$	$71 \pm 2^{d,2}$	$75 \pm 2^{f,3}$	$70 \pm 1^{d,3}$	$45 \pm 2^{b,3}$	$44 \pm 1^{a,2}$	$48 \pm 1^{c,2}$
	Persimmon	$68 \pm 2^{f,1}$	$66 \pm 2^{ef,1}$	$65 \pm 2^{de,1}$	$64 \pm 2^{d,1}$	$60 \pm 2^{c,1}$	$38 \pm 2^{b,1}$	$35 \pm 2^{a,1}$	$36 \pm 2^{a,1}$
S:G-EO-L	Apple	$74 \pm 2^{g,2}$	$67 \pm 3^{f,2}$	$64 \pm 4^{e,2}$	$53 \pm 2^{d,1}$	$53 \pm 4^{d,1}$	$38 \pm 3^{a,1}$	$45 \pm 2^{b,3}$	$50 \pm 2^{c,2}$
	Tomato	$79 \pm 2^{f,3}$	$75 \pm 2^{e,3}$	$73 \pm 2^{d,3}$	$69 \pm 2^{c,3}$	$70 \pm 2^{c,3}$	$49 \pm 3^{b,2}$	$42 \pm 1^{a,2}$	$50 \pm 1^{b,2}$
	Persimmon	$55 \pm 4^{cd,1}$	$57 \pm 3^{de,1}$	$57 \pm 1^{e,1}$	$60 \pm 2^{f,2}$	$55 \pm 2^{c,2}$	$38 \pm 1^{a,1}$	$39 \pm 2^{a,1}$	$41 \pm 2^{b,1}$

Different superscript letters within the same row indicate significant differences among formulations for a determined fruit ($p < 0.05$).

Different superscript numbers within the same column indicate significant differences among fruits for a determined Tween concentration ($p < 0.05$).

Table 5 shows the values of the surface tension of the CFS as a function of the concentrations of Tween 85. It is remarkable that EO, either emulsified or encapsulated, significantly decreased the surface tension of the S:G solutions, leading to values near the critical surface tension of the fruits. Then, no surfactant would be necessary to promote the coating spreading on the fruit surface in these cases. The surface activity of the EO could not be attributed to the amphiphilic nature of the EO components, but to the prevalent location of the EO droplets at the air-liquid interface, due to the hydrophobic nature of the EO dispersed droplets, with greater affinity with the air phase, as occurs in foams stabilised by fat globules (Eisner, Jeelani, Bernhard, & Windhab, 2007). When EO is encapsulated in lecithin liposomes, a similar effect could occur.

In the S:G solution, the surface tension progressively decreased when the Tween concentration rose, but values close to the critical surface tension of the different fruits were only reached at the highest concentration of the surfactant. The decrease in the surface tension at this high concentration could be explained by the interaction of Tween 85 with the amylose molecules, as previously commented on, which limits its surface activity, forming the typical amylose-lipid complexes where the chain of the surfactant is included in the hydrophobic cavities of the amylose helical conformation (Marín, Atarés, Cháfer, & Chiralt, 2017). In fact, some authors report a lipid complexation capacity of amylose in the helical hydrophobic cavity of 10 g lipid/100 g amylose (Eliasson, 1994). Considering an amylose content of 10 % (w/w) of cassava starch (Cano et al., 2014) and the ratios of starch-Tween in the CFS, a total complexation could occur, at lower surfactant levels, without saturating the amylose complexation capacity. This could explain the lack of notable changes in the surface tension at relatively high surfactant concentration.

In contrast, the values of surface tension significantly increased in the S:G-EO system from a Tween 85 concentration of 10^3 mg/L. This behaviour suggests that, at lower concentrations of surfactant, the molecules preferably adsorbed at the oil-water interface, helping to stabilise the EO emulsion. When the oil-water interfacial area was saturated, the surfactant molecules displaced, at least partially, the oil droplets from the air-water interface, thus provoking a notable change in the surface tension. At the highest amounts of Tween, the surface tension is still higher than that of both the Tween-free emulsion and the critical surface tension of the fruits. Therefore, in the S:G-EO formulation, the incorporation of the surfactant did not exert a positive effect on the spreading of the CFS on the fruit. A similar phenomenon occurred in the CFS with the EO encapsulated in lecithin liposomes, where the surfactant-free CFS already had low surface tension. In this particular case, the surfactant molecules could participate in the structure of the liposomes helping to stabilise the oil droplets, thus developing a limited role in the water-air interface. Therefore, the interactions of the surfactant at different levels with the components of the CFS limit its surface activity and its effectiveness in reducing the surface tension.

Table 5. Surface tension (γ_L) values of the different CFS as a function on the Tween 85 concentration (mg/L). Mean values and standard deviations.

Sample	0	10^0	10^1	10^2	10^3	10^4	$5{\cdot}10^4$	10^5
S:G	66.4 ± 0.5^d	67.6 ± 0.4^e	68.0 ± 0.4^f	68.0 ± 0.3^f	67.3 ± 0.2^e	42.5 ± 0.5^c	40.2 ± 0.9^b	17.9 ± 0.5^a
S:G-EO	16.9 ± 0.2^a	17.2 ± 0.2^a	18.5 ± 0.1^b	18.6 ± 0.1^b	42.5 ± 0.4^f	34.9 ± 0.6^e	30.8 ± 0.8^c	31.9 ± 1.2^d
S:G-EO-L	18.4 ± 0.3^a	18.2 ± 0.1^a	18.0 ± 0.2^a	45.7 ± 0.6^f	44.7 ± 1.2^e	39.0 ± 0.7^d	32.8 ± 0.8^b	34.8 ± 0.5^c

Different superscript letters within the same row indicate significant differences associated to the Tween concentration for a determined formulations ($p < 0.05$).

Figure 3 shows the obtained values of the W_a, W_c and W_s for the different fruits as a function of the Tween concentration. Negative values of W_s were obtained in every case, but in practical terms the closer the W_s values are to zero, the better the coating spreading. Therefore, in terms of wettability, the S:G formulation without Tween presented the least favourable behaviour, whereas CFS with emulsified or encapsulated EO exhibited good wettability on the different fruit surfaces, with the closest-to-zero values of W_s. Although Tween incorporation at concentrations higher than 10^3 mg/L enhanced the wettability of S:G systems on the three fruits, it did not improve the wettability of CFS containing EO at any concentration. In fact, a sharp decrease in the W_s of these CFS was observed when Tween was added at 10^2 - 10^3 mg/L, when the surface tension of these CFS increased, as commented on above. Therefore, the S:G solution requires the addition of at least $5{\cdot}10^4$ mg/L of Tween to have a good wettability on the fruit surface, but the CFS with EO, emulsified or encapsulated in lecithin liposomes, did not require the incorporation of Tween 85 to improve their spreading on the fruit. On the contrary, at intermediate concentrations, a highly negative effect was observed.

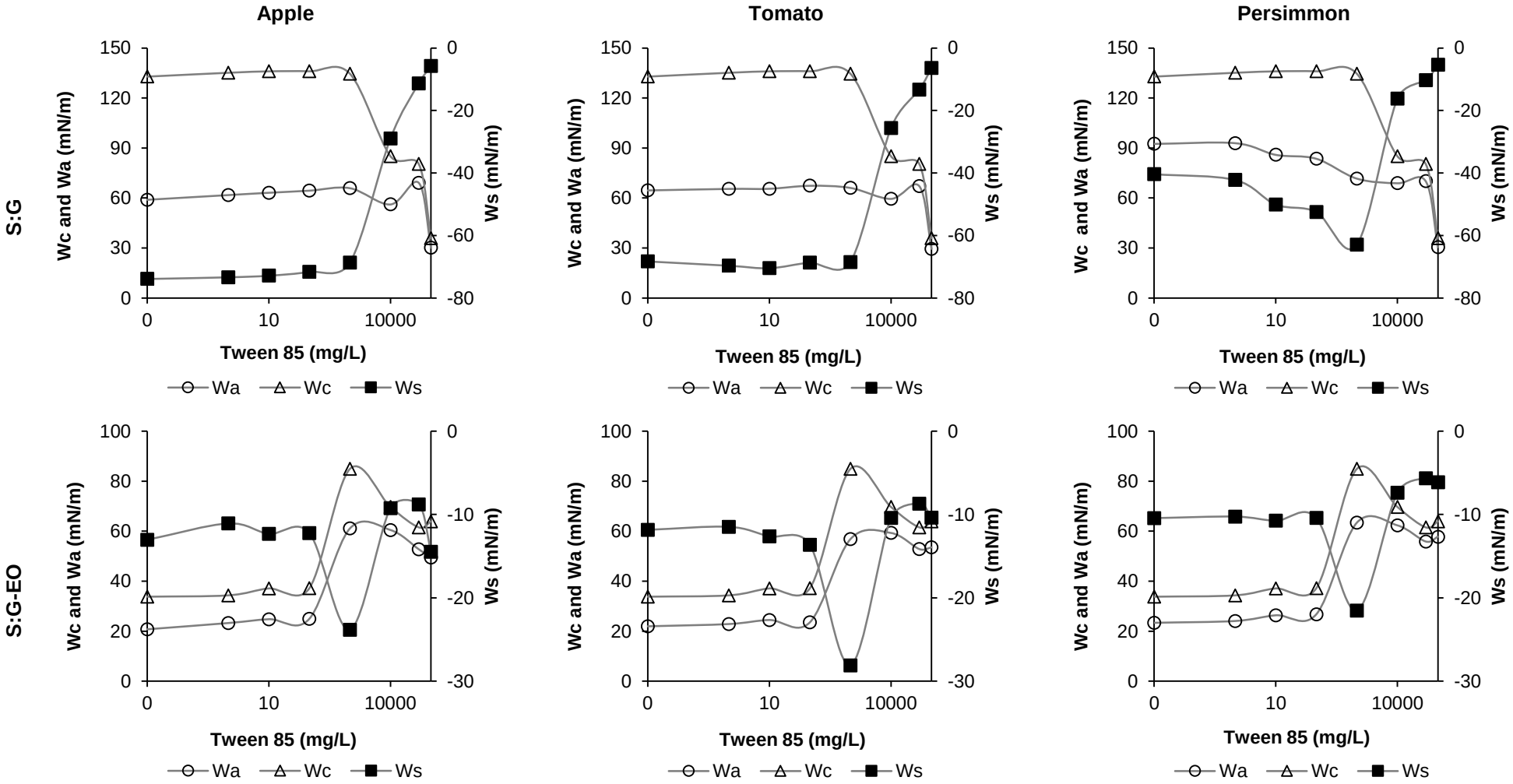

Figure 3. Spreading (W_s), adhesion (W_a) and cohesion (W_c) coefficients obtained for the different CFS, on the apple, tomato and persimmon surfaces, as a function of Tween 85 concentration.

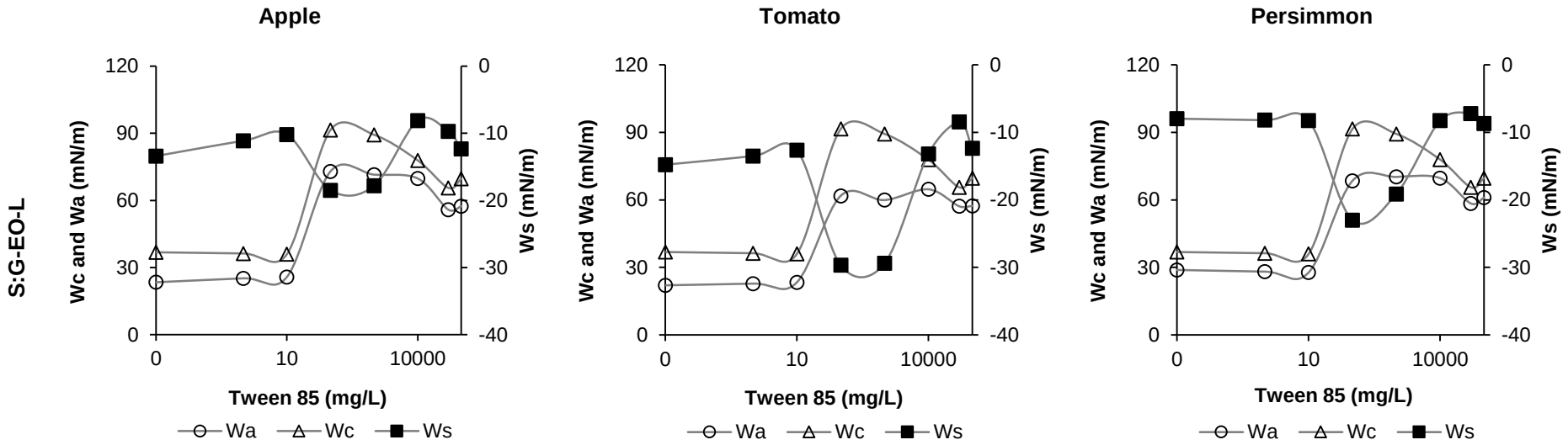

Figure 3. (Continued).

4. CONCLUSIONS

The skins of apple, tomato and persimmon were found to be of low-energy surfaces, their surface tension and critical surface tension values being, respectively, 29.2 and 22.7 mN/m for apple, 21.7 and 16.1 mN/m for tomato, and 25.4 and 20.3 mN/m for persimmon. The polar components were 9.4, 3.6 and 6.0 mN/m, and the dispersive components 19.8, 18.2 and 19.4 mN/m, respectively, for apple, tomato and persimmon, which demonstrates the ability of these surfaces to interact with non-polar liquids. The addition of Tween 85 positively influenced the contact angle and surface tension values of the starch-gellan solutions, but in the presence of thyme essential oil and lecithin, it had a negative impact depending on the concentration of the surfactant. W_s was notably improved with Tween 85 at $5 \cdot 10^4$ mg/L in the S-G formulations (values closer to zero). However, it had a negative effect on the already good spreadability of S:G-EO and S:G-EO-L coatings. These findings provide relevant information on surface properties of starch-gellan coating-forming solutions in view of their use as coatings for fruits. However, it would be necessary to analyse the effect of the coatings with Tween 85 on the preservation parameters of fruits, since this type of compound could also interact with the wax layer of the fruits, modifying their natural barrier capacity.

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CHAPTER 4. Antifungal starch-gellan edible coatings with thyme essential oil for the postharvest preservation of apple and persimmon

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ABSTRACT

Starch-gellan (80:20) coating formulations were applied to apples and persimmons to analyse their effectiveness at controlling weight loss, respiration rate, fruit firmness and fungal decay during postharvest. Thyme essential oil (EO) was incorporated (0.25 and 0.5 g/g of polymer) directly or encapsulated in lecithin to enhance antifungal action. Coatings did not reduce weight loss or firmness changes in apples, but they prevented water loss in persimmons. In contrast, no significant effect of the coatings was observed on respiration rates and respiration quotient of persimmons, whereas they increased the respiration rates and quotient in apples. On the other hand, the coatings without lecithin reduced the incidence and severity of black spot caused by *Alternaria alternata* in persimmons, regardless of the content of essential oil. Likewise, they reduced the severity of grey mould caused by *Botrytis cinerea* in apples. No positive effect of lecithin in coatings was observed on the postharvest quality and decay in either apple or persimmon, nor did EO exert antifungal action despite its proven effectiveness in *in vitro* tests.

Keywords: cassava starch; gellan; thyme essential oil; antifungal edible coatings; postharvest; fruit.

1. INTRODUCTION

Postharvest diseases are one of the major factors that affect the quality of horticultural fresh products during storage. Since fruit and vegetables are living organisms, their shelf life is greatly affected by temperature, relative humidity (RH), composition of the atmosphere during and after harvest, and the type and degree of infection by microorganisms or attack by insects (Singh & Sharma, 2018). Fruit contains high levels of sugars and nutrient elements and the low pH values make them particularly susceptible to fungal decay. Fruit fungal infection may occur during flowering, fruit growth, harvesting, transport, packing operations, postharvest storage, or after purchase by the consumer (Sivakumar & Bautista-Baños, 2014). Moreover, the natural resistance of fruit and vegetables to disease declines with storage duration and ripeness (Troncoso-Rojas & Tiznado-Hernández, 2014).

Grey mould caused by *Botrytis cinerea* Pers. is considered one of the most serious and common postharvest diseases of various fruit, including apples and persimmons (Batta, 2004; Palou, Montesinos-Herrero, Tarazona, Besada, & Taberner, 2015). The infection may occur in the field, from bloom to harvest, or after harvest, typically causing nests of decay. In fruit like apples, that are often stored for extended periods, field infections that remained latent can resume growth during storage, when the pathogen takes advantage of fruit maturity and environmental conditions and the disease develops (low temperatures and high humidity). In this sense, *B. cinerea* is very well adapted to low temperatures and it is even able to grow at 0 ºC (Ma, He, Liu, & Zhou, 2018). Infection starts with a darker circular area where the fruit tissues are softer than the other fruit parts, and subsequent abundant sporification, whose colour ranges from white to grey, can develop from the site of infection in conditions of ambient temperature and high humidity (Romanazzi & Feliziani, 2014). *Alternaria alternata* (Fr.) Keissl. is the causal agent of postharvest black spot in persimmon (*Diospyros kaki* Thunb.) (Prusky et al., 2001), and is generally considered a weak and opportunistic pathogen that gains entry into the fruit via wounds or natural openings, and remains quiescent until the fruit ripens (Biton et al., 2014). *A. alternata* and *Penicillium* spp. were found to be the main causal agents of latent and wound infections in persimmon in Spain (Palou et al., 2015).

After harvest, fresh produce also suffer physiological and biochemical changes that cause detrimental changes in quality and shelf life. Respiration, transpiration and ethylene production are the main factors contributing to the deterioration of fruits and vegetables (Olivas & Barbosa-Cánovas, 2009). Ethylene is a hormone produced by climacteric fruits, or when fruit undergoes stress, and is partially responsible for changes in the flavour, colour and texture of fruits and vegetables. In addition, fresh fruits and vegetables lose water during storage due to respiratory and transpiration processes (Maftoonazad, Ramaswamy, & Marcotte, 2008). Water stress also causes metabolic alterations and changes in enzyme activation, causing accelerated senescence, a decline in nutritional value and increased susceptibility to chilling injury and pathogen invasion. Respiration consists of the oxidative breakdown of organic reserves to simpler molecules, including carbon dioxide (CO_2) and water, with the release of energy (Fonseca, Oliveira, & Brecht, 2002). All these biological factors, such as respiration, ethylene

production and resistance to water diffusion, depend on the fruit commodity and cultivar, physiological stage at harvest, and storage conditions, which are also related to the composition of the surface waxes. Thus, for example, Morice & Shorland (1973) reported that hydrocarbons, alcohols, fatty acids, ursolic acid and α-farnesene are the main components in natural apple surface waxes and the amount and composition of these components changed during storage depending on the apple cultivar.

In the last decade, considerable research has been carried out into the development of edible coatings aiming to control the physiological activity of fruits. These coatings can modify the internal gas composition and reduce the water loss through the regulation of oxygen (O_2), CO_2 and water vapour exchange between the fruit and the surrounding atmosphere. However, a certain degree of O_2 and CO_2 permeability is necessary to avoid anaerobic respiration that induces ethanol production, off-flavour formation and the loss of produce quality (Olivas & Barbosa-Cánovas, 2009). An additional advantage of edible coatings is the possibility of incorporating food-grade ingredients, such as antimicrobial agents, antioxidants, flavours, colour pigments and vitamins, into the basic formulation with the aim of improving their functional properties.

Traditionally, postharvest disease control of fresh fruits and vegetables involves the use of synthetic chemical fungicides in those products for which their use is legislated. However, new restrictive regulations regarding fungicide residues, the reduction of the legal acceptability limits of specific fungicides, the emergence of fungicide resistant strains of the pathogens, and an increasing public concern towards these compounds have led to a global increase in the need to seek safer postharvest alternatives to control the decay of fruits and vegetables (Prusky, Kobiler, Akerman, & Miyara, 2006). Some of these include antimicrobial antagonists (bacteria, yeast, and fungi) that perform as biocontrol agents, synthetic and natural antimicrobials classified as food-grade additives or generally recognized as safe (GRAS) compounds, such as organic and inorganic acids and their salts, chitosan, essential oils (EOs) or other plant extracts and different physical methods. Among the natural compounds, EOs and their components have been reported to suppress fungal growth, both in *in vitro* and *in vivo* studies. Thus, for example, tea tree, palmarosa and star anise EO vapours completely inhibited the *in vitro* germination of the apple pathogen *Penicillium expansum* L. (da Rocha Neto, Navarro, Canton, Maraschin, & Di Piero, 2019); *Melissa officinalis* EO was effective against *B. cinerea*, *P. expansum* and *Rhizopus stolonifer* (Ehrenb.) Vuill. in *in vitro* studies (El Ouadi et al., 2017). *Pulicaria mauritanica* EO was effective against *Alternaria* sp., *P. expansum*, and *R. stolonifer* (Desjobert et al., 2013). In *in vivo* studies, the addition of lemon EO enhanced the antifungal activity of chitosan against *B. cinerea* in strawberries (Perdones, Sánchez-González, Chiralt, & Vargas, 2012); garlic extracts and clove EO treatments reduced the postharvest decay caused by *B. cinerea* and *P. expansum*, when applied directly to apples (Daniel, Lennox, & Vries, 2014); and a chitosan-oregano EO emulsion exhibited an inhibitory effect on pomegranate fruit inoculated with *Botrytis* sp., but caused some phytotoxicity (Munhuweyi, Caleb, Lennox, van Reenen, & Opara, 2017). Usually, the antibacterial effect of EOs relies on their high content of terpenes and terpenoids and also on the content of other

aromatic and aliphatic constituents, all of which are characterized by low molecular weight (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

However, in spite of the great potential of EOs, the main limitation to their application for decay control is the possible induction of a strong odour or flavour in fruit, phytotoxicity risks, and technological issues associated with commercial-scale fumigations or liquid applications (Palou, Ali, Fallik, & Romanazzi, 2016). The addition of EOs to edible coatings based on polymeric matrices could render them more effective at prolonging the postharvest life of horticultural produce, slowing down the diffusion rate of the antimicrobial agent and maintaining a higher concentration of the active compound on the fruit surface for a longer period, while preventing phytotoxicity. Additionally, fruit coatings can delay or retard the ripening process in climacteric fruit by modifying their internal gas composition and changing their permeability to O_2, CO_2 and ethylene production (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011). Among the different EOs, thyme EO exhibited antifungal action against *B. cinerea* and *A. alternata* when included in starch-gellan films in *in vitro* studies (Sapper, Wilcaso, Santamarina, Roselló, & Chiralt, 2018), as well as a complete growth inhibition of *B. cinerea* as vapour in *in vitro* tests (Plotto, Roberts, & Roberts, 2003).

In this study, starch-gellan coatings incorporating thyme (*Thymus zygis* Loefl. ex L.) EO were applied to apples and persimmons to evaluate: (1) the postharvest behaviour of coated fruit in terms of weight loss, respiration rates and mechanical properties, (2) the antifungal efficacy of these coatings applied as a curative treatment against *B. cinerea* in apple and *A. alternata* in persimmon.

2. MATERIALS AND METHODS

2.1. Reagents

To prepare the coating-forming systems (CFS), cassava starch (S) (with 10 % amylose content) (Quimidroga S.A., Barcelona, Spain), low acyl gellan gum (G) (KELCOGEL F, Premium Ingredients, Murcia, Spain), non-GMO soy lecithin with 45 % phosphatidylcholine (L) (Lipoid P45, Lipoid GmbH, Ludwigshafen, Germany) and thyme (*T. zygis*) essential oil (Plantis, Artesanía Agrícola SA, Barcelona, Spain) (EO), were used. The glycerol used as plasticizer was supplied by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain) and the polyoxyethylenesorbitan trioleate (Tween 85®) (T) was purchased from Sigma-Aldrich (Madrid, Spain). Potato Dextrose Agar (PDA) (Scharlab, Barcelona, Spain) was used for the *in vitro* microbial assays.

2.2. Preparation of CFS

The formulations were prepared using S and G in a ratio of 8:2, with glycerol as plasticizer (0.25 g/g of polymer), on the basis of previous studies (Cano et al., 2015; Jiménez, Fabra, Talens, & Chiralt, 2012). Firstly, S was dispersed in distilled water and kept at 95 ºC for 30 min to induce complete starch gelatinization. Meanwhile, G solution was obtained under stirring at 90 ºC for 60 min. Both solutions were cooled down and afterwards, glycerol was added. The S and G systems were mixed to obtain the solutions without EO. The thyme EO (0.25 and 0.5 g/g of polymer), used as antifungal agent, was incorporated, either by direct emulsification or encapsulated in lecithin liposomes (polymer:lecithin ratio of 1:0.5). In the first case, the EO was added directly and the dispersions were homogenized for 3 min at 13.500 rpm using a rotor-stator homogenizer (Ultraturrax Yellow Line DL 25 Basic, IKA, Staufen, Germany). In the second case, the liposome dispersions were previously prepared and added directly to the initial polymer blend solution and kept under soft magnetic stirring for 2 h. A formulation was also obtained with lecithin liposomes without EO, as a control. To obtain the lecithin dispersions, lecithin (5 %, w/w) was dispersed in distilled water and stirred for at least 4 h at 700 rpm. The EO (2.5 % and 5 % w/w) was added to the lecithin dispersion by using a sonicator (Vibra Cell, Sonics & Materials, Inc. USA) at 20 kHz for 10 min with pulses of 1 s, as described by Valencia-Sullca et al. (2016). Tween 85 was also added to S:G CFS (10^5 mg/L) in order to ensure the complete wettability of the fruit surface, according to a previous study (Sapper, Bonet, & Chiralt, 2019) and tested on apples in a preliminary test. All the solutions were degassed by using a vacuum pump (MZ 2C NT, Vacuubrand GmbH + CO KG, Germany). A total of six formulations were obtained: starch: gellan (S:G); control with lecithin (S:G-L); formulations with EO, non-encapsulated (S:G-0.25 and S:G-0.5) and lecithin- encapsulated (S:G-0.25-L and S:G-0.5-L).

2.3. Rheological behaviour and contact angle of the CFS

The rheological behaviour was analysed in triplicate at 25 ºC by means of a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany), by using a sensor system of coaxial cylinders, type Z34DIN Ti. Measurements were taken between 0 and 100 s^{-1}. The obtained data was fitted to the Ostwald de Waale power law model (Eq. (1)) in order to determine the consistency (K) and the flow behaviour indices (n).

$$\sigma = K \cdot \left(\frac{\partial u}{\partial y}\right)^{n} \quad (1)$$

Where σ is the shear stress (Pa), K is the flow consistency index (Pa·s^n), $\frac{\partial_u}{\partial_y}$ represents the shear rate (s^{-1}) and n is the flow behaviour index.

The contact angle (θ) was determined by means of a Dynamic Contact Angle measuring device and Tensiometer (OCA 20, DataPhysics Instruments GmbH, Filderstadt, Germany). For this purpose, thin sections of the skin of the fruit were cut and placed on a glass plate to proceed with the measurements. Then, a droplet of each formulation was placed on the horizontal surface with a needle of 1.19 mm in internal diameter and the contact angle at the fruit surfaces was measured by the sessile drop method (Kwok & Neumann, 1999). Measurements were taken in less than 10 s. Image analyses were carried out using SCA20 software. At least twelve replicates were obtained.

2.4. Quality of coated fruit

Apples (*Malus domestica* Borkh cv. Golden Delicious) and persimmons (*Diospyros kaki* Thunb. cv. Rojo Brillante) were purchased from local packinghouses (Valencia, Spain) before any postharvest treatments were applied. Fruit were chosen according to their uniform shape, size and colour and the absence of surface defects, subsequently cleaned and disinfected by a 4 min immersion in a 1% sodium hypochlorite solution, thoroughly rinsed with tap water, and air-dried at room temperature, before coating application.

CFS were applied manually, using approximately 1.5 mL/fruit, spread evenly over the fruit surface by using latex glove hands, following the method described by Bai, Baldwin, & Hagenmaier (2002). Water was applied to control fruit. Then, each fruit was inspected to assure complete coverage and all fruit were stored at 25 ºC and 65 % RH, for 14 days. Ten fruits were considered in each series (coated and non-coated fruits).

2.4.1. Surface density of solids (SDS)

The SDS was determined by weighing the samples with a precision balance (Kern PFB 120-3, Germany) before and after coating application to obtain the CFS adhered mass. To calculate

the total adhered solids, the mass fraction of each CFS was considered and the SDS (g/m^2) was estimated applying Eq. (2), according to Marín, Atarés, Cháfer, & Chiralt (2017).

$$SDS = \frac{(m_C - m_0) \cdot X_{sCFD}}{m_0} \cdot \rho \cdot \frac{1}{S_e} \quad (2)$$

Where: m_C = mass of the coated apple; m_0 = mass of the uncoated apple; X_{sCFS} = mass fraction of the solids of the CFS (g solids/g solution); ρ = apple density (g/cm^3). To obtain the specific surface (S_e = 6/d, m^2 surface/m^3 fruit), the average diameter (d) was calculated considering a spherical geometry for the fruit.

2.4.2. Weight loss

The weight loss of the fruit during storage was measured using an analytical balance (ME235P Sartorius, Germany) before and after 3, 7 and 14 days of storage. The mass loss was referred to the initial mass of the fruit, and the results were expressed as relative mass loss rate ($days^{-1}$), obtained from the slope of the fitted straight line to the relative weight loss *vs.* time data. Ten fruits were considered for each formulation and for control fruit.

2.4.3. Respiration rates

Measurements were taken using a closed system, following the method proposed by Castelló, Fito, & Chiralt (2010), with some modifications. Two apples were placed in hermetic glass jars with a septum in the lid for sampling headspace gas at different times. Gas sampling was carried out every 30 min for 4 h by means of a needle connected to a gas analyser (CheckMate 9900 PBI Dansensor, Ringsted, Denmark). Three replicates per treatment were performed after 7 and 14 days of storage. The respiration rate (R_i) of the samples in terms of CO_2 generation and O_2 consumption was determined from the slope of the fitted linear equation, according to Eq. (3). The respiration quotient (RQ) has been determined as the ratio between CO_2 production and the O_2 consumption.

$$y_{it} = y_{i0} \pm 100 \cdot R_i \cdot \frac{M}{V} \cdot t \quad (3)$$

Where y_{it} is the gas concentration (%O_2, %CO_2) at time *t*, y_{i0} is the initial gas concentration, R_i is the respiration rate (mL/kg h), M is the mass of the samples, V the volume (mL) of headspace and t is the time.

2.4.4. Fruit firmness

The firmness was measured using a Texture Analyser (Stable Micro Systems, TA.XT plus, Haslemere, England) fitted with an 11 mm diameter probe, applying a modification of the method described by Saei, Tustin, Zamani, Talaie, & Hall (2011). A small skin area was removed from four opposite sides of each fruit around the equator. The probe penetrated the

flesh at 10 mm/ min and the maximum force (F_{max}, N) required to break the flesh was used as fruit firmness. The distance at maximum force (d_{max}, mm) was also taken as another representative parameter of the puncture curve. Ten replicates were used for each formulation, after 14 days of storage. The same procedure was applied to uncoated fruit (control), both at the beginning and after 14 days of storage.

2.5. In vivo antifungal assays

For the *in vivo* assays, *B. cinerea* strain BC03 from the IRTA Culture Collection (Lleida, Catalonia, Spain) was originally isolated from infected grapes from a vineyard located in Lleida (Catalonia, Spain) and it was deposited at the Spanish Type Culture Collection (CECT-20973) at the University of Valencia (Burjassot, Valencia, Spain). An *A. alternata* strain QAV-6 had been isolated from decayed persimmon fruit and maintained in the IVIA CTP Culture Collection of postharvest pathogens (Moncada, Valencia, Spain). These fungal strains were cultured on Potato Dextrose Agar (PDA) petri dishes at 25 ˚C in the dark and used after 7-14 days of active growth. Conidia were scraped from the cultures using a sterile loop and subsequently filtered and transferred to test tubes with sterile distilled water and 0.01% Tween 85. The suspensions were adjusted at $1x10^4$ conidia/ mL for *B. cinerea* and $5x10^5$ conidia/ mL for *A. alternata.* The concentration of conidial suspensions was determined using a haemocytometer.

Fruit were wounded (approximately 1.6 mm in diameter and 2 mm deep) using the tip of a stainless-steel rod once in the fruit equator in the case of apples, and twice in the equator on the same side of the fruit in the case of persimmons (wounds located midway between the calyx and the stem end and 5-6 cm apart). Each wound was inoculated using a micropipette with 20 µL of the correspondent spore suspension 24 h before the application of the coatings (assessment of the coatings' curative activity). As previously described, coatings were applied manually at approximately 1.5 mL/fruit. Air surface drying was allowed at room temperature and fruit were subsequently placed in perforated plastic trays avoiding direct contact between fruit and incubated at 20 ˚C and 85 ± 5 % RH. Twenty fruits, four replications of five fruit each were used per treatment. Control fruit were inoculated and treated with water using the same procedure as that for coating application. Lesion diameters (disease severity, mm) were measured after 7 and 12 days of incubation. Disease incidence (%) was expressed as the percentage of infected wounds out of the total number of inoculated wounds per replicate and treatment.

2.6. Statistical analysis

The statistical analyses of the results were performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVI.II (StatPoint Technologies Inc., Warrenton, VA, USA). Fisher's least significant difference (LSD) test was used at the 95% confidence level to determine specific differences between means. Multifactor ANOVA was also used to analyse the effect of the different factors (storage time and type of coating).

3. RESULTS AND DISCUSSION

3.1. CFS properties

Viscosity and contact angle of the different CFS on apple and persimmon skin were analysed since these parameters can affect the coating retention/adhesion on the fruit surface after the coating treatment, through their influence on the CFS gravitational drainage before drying and liquid spreadability, all of which affects the coating thickness and homogeneity. Flow curves of the CFS were fitted to the Power Law model and the rheological parameters (consistency index: K and flow index: n), including the apparent viscosity (η) at 100 s^{-1}, are shown in Table 1. A pseudoplastic behaviour, with similar values of n, lower than 1, was observed in all cases. Apparent viscosities ranged between 25 and 42 mPa.s, depending on the CFS composition. Directly-emulsified EO caused an increase in the apparent viscosity of the CFS according to the EO ratio, while lecithin-encapsulated EO reduced the viscosity of the formulations, probably due to the smaller droplet size in the encapsulated system (Valencia-Sullca et al., 2016). Thus, the S:G-0.5 sample was the most viscous formulation and showed the highest consistency index.

The contact angles of the different CFS on apple and persimmon skin are also shown in Table 1. Values lower than 90˚ indicate surface wettability and, therefore, greater extensibility of the coating on the fruit surface. For a given CFS, the contact angles on the persimmon skin were lower than on the apple skin, which indicates a better wettability of persimmon with these types of formulations. The values depended on the coating composition, with the highest contact angle corresponding to the S:G formulation in apples. This could imply problems for the extension of this coating on the apple surface. A previous study (Sapper et al., 2019) reported that 10^5 mg/L of Tween 85 must be added to ensure the S:G coating spreadability on the apple surface, whereas no surfactant was necessary to enhance the CFS spreadability when they contained emulsified or lecithin-encapsulated EO. Therefore, Tween 85 was added to the S:G formulation and tested in a preliminary trial with apples, in comparison with the CFS without Tween 85, in order to analyse the effect of the surfactant on the fruit quality during storage, as discussed in the next section.

Table 1. Rheological parameters (flow behaviour index, n; consistency index, K; apparent viscosity at 100 s^{-1}, η) and contact angle (θ) of the coating forming solution (CFS) on the skin of 'Golden Delicious' apple and 'Rojo Brillante' persimmon. Mean values and standard deviations.

CFS	Rheological behaviour			Contact angle (θ)	
	n	K (mPa.s)^n	η at 100 s^{-1} (mPa·s)	Apple	Persimmon
S:G	0.854 ± 0.001^d	65.0 ± 0.2^a	33.1 ± 0.1^c	96 ± 2^e	67 ± 3^{cd}
S:G-L	0.74 ± 0.01^a	114 ± 3^b	35.0 ± 0.1^d	85 ± 3^d	72 ± 2^e
S:G-0.25	0.86 ± 0.01^d	59 ± 9^a	31 ± 3^b	69 ± 3^a	65 ± 3^c
S:G-0.25-L	0.815 ± 0.001^c	59.7 ± 0.5^a	25.5 ± 0.3^a	73 ± 2^b	50 ± 6^a
S:G-0.5	0.766 ± 0.004^b	124 ± 3^c	42.2 ± 0.2^e	77 ± 2^c	68 ± 2^d
S:G-0.5-L	0.809 ± 0.002^c	60 ± 1^a	25.05 ± 0.03^a	74 ± 2^b	55 ± 4^b

Different superscript letters within the same column indicate significant differences among CFS according to Fisher's LSD test ($P < 0.05$).

3.2 Effect of the incorporation of Tween 85 into CFS on apple quality

The incorporation of Tween 85 into the S:G CFS significantly decreased the contact angle on the apple surface (from 96 ± 2 to 47 ± 3) and increased the apparent viscosity (from 37.9 ± 0.3 to 187.8 ± 0.1 mPa s). As expected, both changes affected the retention/adhesion of the CFS on the apple surface as shown in the values of SDS on the fruit, which ranged from 2.6 ± 0.8 to 3.4 ± 0.5 g/m^2. Interactions of Tween 85 with the CFS components and with the fruit surface affected both the viscosity and the contact angle of the CFS. As described by Marín et al. (2017), surfactant molecules form complexes with the helical conformation of amylose favouring the chain aggregation and increasing the system viscosity. Likewise, this complex formation implies that a high amount of surfactant is required to enhance the spreading of the CFS on the fruit surface, as discussed by Sapper et al. (2019). The increase in the SDS values for the S:G formulation with Tween 85 can be attributed to the higher solid content of the formulation, the greater viscosity that limits liquid gravitational drainage and the lower contact angle. However, given the amphiphilic nature of the surfactant, its interactions with the natural wax of the fruit cuticle could also modify the overall barrier properties of the wax-coating assembly on the fruit surface. As is known, cuticular waxes are the primary components of the cuticle responsible for its permeability and wettability. These waxes are embedded in the cutin and form a continuous layer on the top of the cutin (Belding, Blankenship, Young, & Leidy, 1998). It has been reported that the cuticular wax content in apple fruit increases during fruit development and storage (Ju & Bramlage, 2001).

Table 2 shows the relative weight loss rate, respiration rate and puncture parameters of apples after 7 days of storage at 25 ºC for samples coated with the S:G formulation containing or not Tween 85, in comparison with the uncoated control sample. Little differences in relative weight

loss rate were observed between the uncoated control sample and the one coated with the surfactant-free formulation. However, significantly higher weight loss rate was observed for those coated with the formulation containing Tween 85. The coatings reduced the O_2 consumption rate of the fruit, which can be attributed to the low O_2 permeability of these films (Sapper et al., 2018), but this reduction was particularly significant for the coating containing Tween 85. The CO_2 production rate was not significantly affected by the S:G coating compared to the control sample, but the coating containing Tween 85 significantly reduced this rate. As a consequence, the respiratory quotient was higher than 1 for both coated samples, indicating the creation of a modified atmosphere in the fruit and a shift towards anaerobic respiration pathways. The incorporation of Tween 85 resulted in a general decrease in the gas transfer rate and an increase in water transfer rate. As discussed above, the interactions of Tween 85 with the cuticular waxes, as well as its effect on the decrease in the cohesion forces of the S:G matrix (limiting of chain packing), could explain the changes observed in the gas and water vapour barrier properties of the coating and their effect on the fruit. These changes also had an effect on the fruit texture as shown in Table 2. Although all the samples exhibited similar fruit firmness as deduced from the lack of significant differences among treatments as regards the maximum puncture force, there were significant differences in the maximum penetration distance (d_{max}) at the tissue rupture. Fruits coated with the S:G formulation containing Tween 85 had significantly higher d_{max} values, which reflects changes in the tissue texture. This fact can be related to the greater loss of water and, therefore, of cellular turgidity, associated with a more marked superficial dehydration of the fruit with this coating. This factor is considered one of the main causes of texture changes in fruits (Saberi et al., 2018). After 7 days of storage, all the samples had higher d_{max} than the fruit at the initial time, those coated with the CFS containing Tween 85 being significantly more deformable. Therefore, the use of Tween in the S:G formulation to improve its wettability on the apple surface was discarded on the basis of the negative effects on the fruit weight loss and texture.

Table 2. Effect of the incorporation of Tween 85 into S:G coating formulation on the postharvest behaviour and quality of coated 'Golden Delicious' apples: relative weight loss rate ($days^{-1}$), respiration rates (consumption of O_2 and production of CO_2, mL/kg h), respiration quotient (RQ) and values of the maximum puncture force (F_{max}, N) and penetration distance (d_{max}, mm) after 7 days of storage at 25 ºC. Uncoated samples were used for values at harvest and the control after 7 days of storage.

	CONTROL	CONTROL	S:G	S:G-Tween 85
	Initial time		7 days	
Weight loss rate	-	0,36 ± 0,02^a	0,36 ± 0,01^a	0,66 ± 0,06^b
F_{max}	43 ± 7	46 ± 6^a	49 ± 8^a	46 ± 4^a
d_{max}	3.0 ± 0.3	3,6 ± 0,4^a	3,9 ± 0,5^a	4,6 ± 0,7^b
R O_2	12.94 ± 0.05	12.9 ± 1.3^b	11.4 ± 0.8^b	7.77 ± 0.02^a
R CO_2	13.9 ± 0.6	15.2 ± 0.7^b	18 ± 1^c	11.0 ± 0.2^a
RQ	1.07 ± 0.05	1,18 ± 0,07^a	1,58 ± 0,03^c	1,41 ± 0,03^b

Different superscript letters within the same row indicate significant differences among formulations according to Fisher's LSD test ($P < 0.05$).

3.3. Effect of CFS on postharvest behaviour and quality of apples and persimmons

Table 3 shows the initial values of respiration rates and puncture parameters of apples and persimmons and Table 4 shows the same parameters, together with the values of SDS, for coated and uncoated 'Golden Delicious' apples and 'Rojo Brillante' persimmons after storage at 25 ºC. SDS values are indicators of the coating thickness on the fruit; the higher the SDS, the thicker the coating. The SDS value depends on the amount of CFS adhered to the surface of the fruit and the total solid content of the formulation. The former, in turn, is affected by the wetting/spreading capacity and the viscosity of the coating formulations. In general, the SDS values were higher in apple than in persimmon, which could be related to differences in both the surface tension of the skin (Sapper et al., 2019) and in the skin morphology of the fruit. Thus, persimmons are characterized by a smooth skin, where the lack of small superficial pores could limit the capillary retention of the liquid fraction. Similarly, the CFS composition slightly affected the SDS differently depending on the fruit. In apple, the presence of lecithin in the formulation significantly reduced the SDS, whereas smaller differences associated with the CFS composition were observed in persimmon, and these were seemingly more closely related to the solid content of the CFS (incorporation of EO and/or lecithin to the formulations).

The rate of relative weight loss after 14 days of storage was not significantly affected by coating application or composition and ranged between 0.20-0.23 day^{-1}. In persimmons, water loss rates were higher than in apples and varied depending on the coating formulation. The highest values (0.7 day^{-1}) were obtained for uncoated samples and those coated with CFS containing the highest content of lecithin-encapsulated EO (maximum lipid content in the film) and the lowest value (0.52 day^{-1}) was obtained for samples coated with the formulation with emulsified

EO (without lecithin) at the lowest ratio (minimum lipid content in the film). No significant differences were found between the other coating formulations and the control samples. These results indicate that persimmon fruits were more sensitive to dehydration than apples under these storage conditions and the coatings with the lowest ratio of emulsified EO exerted a protective effect. As mentioned above, apples and persimmons are naturally covered by a continuous wax layer that provides the resistance to water movement across the cuticle. The differences in water resistance of untreated fruits can be attributed to the particular fruit physiology, skin morphology and the composition of the natural waxes. The application of coatings containing hydrophobic compounds should improve the moisture resistance of the fruit as an additional layer is deposited over the natural waxes. In the present study, none of the coatings reduced weight loss in apples and only the coating that had the lowest amount of EO and no lecithin prevented water loss in persimmons. This might indicate a partial removal and/or modification of the natural waxes present on the peel of the fruits resulting in no reduction in weight loss; so, further studies should be conducted to understand the effect of the EO and lecithin on the water barrier properties of coated apples and persimmons. Some other studies also reflected no effect of coatings based on biopolymers and lipids on weight loss reduction of different fruits, such as apple (Bai et al., 2002), plums (Navarro-Tarazaga, Sothornvit, & Pérez-Gago, 2008), table grapes (Pastor et al., 2011), or cherry tomatoes (Fagundes, Palou, Monteiro, & Pérez-Gago, 2015) compared to uncoated fruits.

Table 4 also shows respiration rates of both fruit at 7 and 14 days of storage. In apples, a multifactor ANOVA (results not shown) did not reveal a significant effect of the storage time on respiration rates, although the coatings had a significant influence. In general, coatings tend to increase the O_2 consumption and CO_2 production rates with respect to the control sample, and only those containing lecithin-encapsulated EO showed no significant differences with respect to the control sample. The alterations in the respiration pathway affected the RQ, which indicates the nature of the substrate used during the respiration process. Thus, a RQ equal to 1.0 indicates that the metabolic substrates are carbohydrates, whereas RQ higher than 1 indicates that the substrates are organic acids (Fagundes, Carciofi, & Monteiro, 2013). The multifactor ANOVA in RQ reveals significant effect of storage time and coating formulation. RQ slightly increased at 14 d and was higher in all coated samples. This indicates that the metabolic substrates are shifting from carbohydrates to organic acids (Fagundes et al., 2013), more quickly in coated samples.

In contrast, coatings were observed to have no significant effect on the respiration rates of persimmons, which exhibited lower respiration rates than apples, with a respiration quotient of nearly 1. Climacteric fruit, like apples, exhibit a peak of respiration and ethylene (C_2H_4) production associated with senescence or ripening (Fonseca et al., 2002), which could explain the observed differences.

The texture changes in fruits depend on both cell wall degradation and the loss of tissue turgidity (Ribeiro, Vicente, Teixeira, & Miranda, 2007). Table 4 shows the values of the F_{max} and d_{max} for the different coated and uncoated fruits after 14 days of storage. No significant changes in the maximum puncture force (failure point) were observed for either uncoated or

coated apples after storage with respect to the initial values before storage (Tables 3 and 4). However, although no significant differences were observed in terms of maximum penetration distance between coated and control apples at the end of the storage, the values were slightly higher than before storage, which can be associated with a loss of cellular turgidity due to the superficial dehydration of the apples. The limited water vapour barrier capacity of these films (Sapper et al., 2018) and their relative lack of thickness on the fruit mean that they are scarcely effective at controlling moisture transfer in apple.

In the case of persimmons, the coatings had a significant effect ($P < 0.05$), maintaining the firmness of the fruit. On the other hand, although there were no notable differences in terms of the maximum penetration distance at the failure point (5-6 mm) between coated and uncoated samples at the end of the storage, the values were significantly higher than the initial value (2.5 mm) before storage. This indicates changes in the texture of the tissue over time, which can be related with the progress in maturity and water loss. The F_{max} values increased from 21.1 up to 31 N, which could be attributed to the greater deformability of the tissue allowing for deeper penetration without failure, thus accumulating more compressive and shear resistance (Harker et al., 2019). The smallest changes occurred in the sample coated with CFS containing lecithin without EO.

The above results show that coatings have a different effect depending on whether the fruit is apple or persimmon which can be attributed to the different physiological patterns of the fruits and the specific interactions with the coatings. Although respiration patterns were slightly modified by coatings on apples with no effect on water loss, coatings exerted a better control of water loss in persimmon; however, they did not maintain the firmness mainly due to the progress of fruit ripening.

Table 3. Respiration rates (consumption of O_2 and production of CO_2, mL/kg h), respiration quotient (RQ) and maximum puncture force (F_{max}, N) and penetration distance (d_{max}, mm) of uncoated 'Golden Delicious' apples and 'Rojo Brillante' persimmons at initial time. Mean values and standard deviations.

	R O_2	R CO_2	RQ	F_{max}	d_{max}
Apple	13.7 ± 1.5	12.2 ± 0.2	0.9 ± 0.1	29 ± 2	2.2 ± 0.1
Persimmon	5.7 ± 1.1	5.4 ± 0.9	0.94 ± 0.02	21.1 ± 0.5	2.5 ± 0.3

Table 4. Surface density of solids (SDS, g/m^2), weight loss rate (g days^{-1}), respiration rates (consumption of O_2 and production of CO_2, mL/kg h), respiration quotient (RQ), maximum puncture force (F_{max}, N), and penetration distance (d_{max}, mm) of coated and uncoated 'Golden Delicious' apples and 'Rojo Brillante' persimmons for 7 or 14 days of storage at 25 ºC.

	CONTROL	S:G	S:G-L	S:G-0.25	S:G-0.25-L	S:G-0.5	S:G-0.5-L
APPLE							
SDS	-	1.3 ± 0.3^{c}	0.8 ± 0.1^{a}	1.4 ± 0.3^{c}	1.1 ± 0.1^{b}	1.5 ± 0.2^{c}	1.0 ± 0.1^{b}
Weight loss rate 14 d	0.23 ± 0.03^{a}	0.21 ± 0.03^{a}	0.20 ± 0.05^{a}	0.21 ± 0.03^{a}	0.20 ± 0.03^{a}	0.20 ± 0.03^{a}	0.22 ± 0.03^{a}
R O_2 7d	6.0 ± 1.4^{ab}	6.0 ± 1.5^{ab}	6.5 ± 1.6^{ab}	6.6 ± 0.8^{ab}	6.5 ± 1.0^{ab}	7.6 ± 0.6^{b}	5.1 ± 0.9^{a}
R CO_2 7d	6.5 ± 1.2^{a}	7.8 ± 0.8^{ab}	8.4 ± 2.0^{ab}	8.8 ± 0.1^{ab}	8.3 ± 0.7^{ab}	9.7 ± 1.0^{b}	6.8 ± 0.9^{a}
RQ 7d	1.1 ± 0.1^{a}	1.3 ± 0.1^{b}	1.29 ± 0.04^{b}	1.4 ± 0.1^{b}	1.3 ± 0.1^{b}	1.3 ± 0.1^{b}	1.3 ± 0.1^{b}
R O_2 14 d	5.3 ± 0.8^{a}	8.4 ± 1.1^{d}	7.3 ± 0.5^{cd}	7.0 ± 0.1^{bcd}	5.9 ± 0.8^{ab}	6.5 ± 0.9^{abc}	5.6 ± 0.9^{ab}
R CO_2 14 d	6.2 ± 1.3^{a}	10.4 ± 1.1^{d}	9.0 ± 0.9^{cd}	8.1 ± 0.2^{bc}	7.2 ± 0.6^{ab}	7.9 ± 0.6^{bc}	7.2 ± 0.6^{ab}
RQ 14 d	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	1.23 ± 0.05^{a}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	1.3 ± 0.1^{a}
F_{max} 14 d	27 ± 2^{ab}	31 ± 3^{c}	30 ± 4^{bc}	29 ± 3^{abc}	30.4 ± 1.5^{bc}	32 ± 2^{c}	26.1 ± 1.2^{a}
d_{max} 14 d	3 ± 1^{a}	3 ± 1^{a}	3 ± 1^{a}	3 ± 0^{a}	3 ± 1^{a}	3 ± 1^{a}	3.4 ± 0.5^{a}
PERSIMMON							
SDS	-	0.5 ± 0.1^{a}	0.7 ± 0.1^{ab}	0.7 ± 0.2^{b}	0.8 ± 0.1^{bc}	0.9 ± 0.1^{c}	0.7 ± 0.2^{b}
Weight loss rate 14 d	0.7 ± 0.1^{b}	0.6 ± 0.1^{ab}	0.6 ± 0.1^{ab}	0.52 ± 0.03^{a}	0.56 ± 0.06^{ab}	0.6 ± 0.1^{ab}	0.7 ± 0.1^{b}
R O_2 7 d	5.2 ± 0.3^{a}	3.3 ± 1.6^{a}	3.8 ± 0.6^{a}	2.9 ± 0.2^{a}	3.9 ± 2.1^{a}	3.6 ± 0.3^{a}	4.1 ± 1.7^{a}
R CO_2 7 d	5.6 ± 0.5^{a}	3.8 ± 1.0^{a}	4.2 ± 0.9^{a}	3.4 ± 0.4^{a}	4.5 ± 2.0^{a}	4.6 ± 0.7^{a}	4.8 ± 2.1^{a}
RQ 7 d	1.08 ± 0.04^{a}	1.2 ± 0.3^{a}	1.11 ± 0.05^{a}	1.19 ± 0.03^{a}	1.3 ± 0.3^{a}	1.3 ± 0.1^{a}	1.2 ± 0.1^{a}
R O_2 14 d	3.7 ± 0.6^{a}	4.2 ± 1.8^{a}	3.0 ± 1.1^{a}	3.3 ± 0.4^{a}	2.2 ± 0.2^{a}	2.6 ± 0.6^{a}	3.5 ± 1.9^{a}
R CO_2 14 d	3.9 ± 1.1^{a}	5.3 ± 2.6^{a}	4.1 ± 1.0^{a}	3.9 ± 1.8^{a}	2.2 ± 0.2^{a}	2.9 ± 0.4^{a}	4.2 ± 1.3^{a}
RQ 14 d	1.0 ± 0.1^{a}	1.3 ± 0.1^{a}	1.4 ± 0.2^{a}	1.2 ± 0.4^{a}	1.0 ± 0.0^{a}	1.1 ± 0.1^{a}	1.3 ± 0.3^{a}
F_{max} 14 d	21 ± 4^{a}	27 ± 3^{bc}	23 ± 4^{ab}	27 ± 5^{bc}	28 ± 6^{c}	29 ± 6^{c}	28 ± 6^{c}
d_{max} 14 d	5 ± 1^{ab}	6 ± 1^{bc}	5 ± 1^{a}	5 ± 1^{a}	5.2 ± 0.4^{abc}	6 ± 1^{c}	6 ± 1^{bc}

Different superscript letters within the same row indicate significant differences among formulations according to Fisher's LSD test ($P < 0.05$).

3.4. Fungal decay

Table 5 shows the development of fungal decay on artificially inoculated 'Golden Delicious' apples and 'Rojo Brillante' persimmons. The applications of starch-gellan coatings did not significantly reduce the disease incidence on apples inoculated with *B. cinerea*, as compared to non-coated ones (control) after 7 or 12 days of storage at 20 ºC. No effect of the addition of EO was observed, despite what had been observed in a prior *in vitro* study, where starch-gellan films with thyme EO exhibited a marked antifungal effect (Sapper et al., 2018). Nevertheless, all coatings, regardless of their composition, significantly reduced the severity of grey mould with respect to the control samples (20-30 % reduction), with no particular observed effect of the antifungal EO.

Starch-gellan coatings were more effective at reducing the incidence of black spot caused by *A. alternata* on persimmon (up to 40% reduction), although coatings containing lecithin were not effective and the presence of EO was not observed to have any significant effect. Disease severity was not significantly reduced in coated persimmons. A multifactorial analysis (factors: presence of lecithin and EO concentration) revealed two things: there was no significant

influence of the EO and the lecithin had a negative effect on the reduction of disease incidence and severity in infected fruit.

In general, applying a coating had a positive antifungal effect both on apples (a significant reduction in the severity of grey mould) and persimmons (a significant reduction in the incidence of black spot), but this antifungal effect was milder than that observed in *in vitro* work with EO incorporated into the same type of films. Similar behaviour has recently been reported by da Rocha Neto et al. (2019) for apples. They observed a complete inhibition of the *in vitro* germination of *P. expansum* by using melaleuca, palmarosa and star anise EOs in vapour phase, but a minor effect of these treatments on inoculated apples, regardless of the EO used. As previously reported (Palou et al., 2016; Tripathi, Dubey, Banerji, & Chansouria, 2004), this indicates that the *in vivo* effectiveness of EOs cannot be anticipated by their antifungal activity in *in vitro* tests and that interactions between EOs and fungal pathogens are modulated by the fruit host and the conditions in the infection court often resulting in reduced disease control ability. An important difference in the potential effect of EO with respect to *in vitro* tests could be related with the degree of coating plasticization that may affect the release of EO. In *in vitro* tests, films are directly applied on the wet culture medium, whereas coatings are applied on the dried fruit surface. This fact could limit the release of the active compounds from the polymer matrix, hindering their antifungal action. Likewise, EO compounds may also affect some physiological changes in the fruit, which could decrease the fruit's natural defences against the fungal attack. The generally negative effect of lecithin could also be attributed to the lipid interactions with the fruit's waxy coatings, which could also weaken the natural resistance to disease, counteracting the induced coating protection. Other surfactant lipids, such as Tween 85, also seemed to exert a negative effect on the barrier capacity of the natural wax-coating assembly, as observed in apples. The gas exchange on the fruit surface could also play an important role in postharvest disease development, which could explain the generally positive effect of coatings at reducing fungal growth on infected fruit. Therefore, interactions of coatings and their components with the fruit surface always constitute a distinguishing factor to define the particular behaviour of coated fruit (Basiak, Linke, Debeaufort, Lenart, & Geyer, 2019), and in the case of coatings formulated with antifungal ingredients, these interactions can affect the *in vivo* disease control ability of the coating (Valencia-Chamorro, Palou, del Río, & Pérez-Gago, 2011). The results obtained in the present study with the addition of thyme EO to starch-based coatings were not anticipated. Numerous previous studies have shown that the formulation of antifungal films and coatings with EOs either provided disease control ability or increased that of the coating alone due to an important synergistic effect against various important postharvest pathogens, including *B. cinerea* and *A. alternata* (Campos-Requena et al., 2017; Grande-Tovar, Chaves-Lopez, Serio, Rossi, & Paparella, 2018; Perdones et al., 2012). However, this is not always the case and other reports showed no significant benefit gained from the addition of EOs (Shao et al., 2015). It seems clear, therefore, that a wide variability in disease control efficacy can be observed, basically due to the numerous factors that can influence the antifungal properties of films and coatings. The following can be cited among the most important: nature of the composite matrix of the coating; type and concentration of the antifungal compound(s); species and strain of the target

postharvest pathogen; species, cultivar and physical and physiological condition of the fruit host; and postharvest environmental conditions.

Table 5. Mean values and standard deviations of disease incidence and severity of gray mould on 'Golden Delicious' apples artificially inoculated with *Botrytis cinerea* and black spot on 'Rojo Brillante' persimmons artificially inoculated with *Alternaria alternata*. Fruit were coated 24 h after fungal inoculation and incubated at 20 ºC and 85 % RH for 7 and 12 days. Mean values of the reduction in disease incidence and severity are also shown.

	Disease incidence (%)		Reduction of incidence (%)		Disease severity (mm)		Reduction of severity (%)	
	7 days	12 days	7 days	12 days	7 days	12 days	7 days	12 days
Apple gray mould								
CONTROL	100 ± 0^{a}	100 ± 0^{a}	-	-	70 ± 5^{b}	100 ± 5^{b}	-	-
S:G	75 ± 25^{a}	83 ± 14^{a}	25	17	44 ± 5^{a}	74 ± 13^{a}	32	26
S:G-L	92 ± 14^{a}	92 ± 14^{a}	8	8	47 ± 11^{a}	73 ± 21^{a}	27	27
S:G-0.25	75 ± 25^{a}	83 ± 14^{a}	25	17	53 ± 8^{ab}	76 ± 15^{a}	19	24
S:G-0.25-L	75 ± 25^{a}	75 ± 25^{a}	25	25	44 ± 11^{a}	64 ± 17^{a}	33	36
S:G-0.5	92 ± 14^{a}	92 ± 14^{a}	8	8	45 ± 10^{a}	81 ± 7^{ab}	32	19
S:G-0.5-L	100 ± 0^{a}	100 ± 0^{a}	0	0	47 ± 1^{a}	69 ± 10^{a}	29	31
Persimmon black spot								
CONTROL	68 ± 3^{b}	73 ± 5^{bc}	-	-	10.6 ± 0.8^{a}	21.9 ± 1.9^{b}	-	-
S:G	38 ± 9^{a}	45 ± 12^{a}	44	39	9.3 ± 1.5^{a}	14.7 ± 3.7^{a}	12	32.9
S:G-L	70 ± 10^{b}	78 ± 7^{bc}	0	0	9.7 ± 1.2^{a}	17.4 ± 2.6^{a}	9	20.8
S:G-0.25	42 ± 7^{a}	57 ± 10^{ab}	39	23	10.8 ± 1.1^{a}	15.5 ± 1.4^{a}	0	29.4
S:G-0.25-L	58 ± 8^{ab}	72 ± 6^{bc}	14	2	12.7 ± 0.6^{a}	18.8 ± 1.9^{ab}	0	14.2
S:G-0.5	42 ± 7^{a}	53 ± 7^{ab}	39	27	11.4 ± 0.3^{a}	20.2 ± 0.5^{ab}	0	8
S:G-0.5-L	72 ± 7^{b}	82 ± 8^{c}	0	0	10.4 ± 0.8^{a}	17.6 ± 0.6^{a}	2	20

For each disease, different superscript letters within the same column indicate significant differences among formulations according to Fisher's LSD test ($P < 0.05$).

4. CONCLUSIONS

Starch-gellan coatings, containing or not emulsified or lecithin-encapsulated EO, applied on apples and persimmons had a different effect on the postharvest parameters (weight loss, respiration rates and firmness changes), depending on the coating composition and type of fruit. None of the coating formulations reduced the weight loss in apples, although they prevented water loss in persimmons. In contrast, although the coating was not observed to have any significant effect on the respiration rates and respiration quotient of persimmons, the respiration rates and quotient in apples were promoted. Coatings did not affect the changes in fruit firmness in apples or persimmons; nevertheless, in the latter these may be mainly associated with the ripening progress. Regarding fungal decay, coatings without lecithin reduced the incidence of black spot caused by *A. alternata* in persimmons, regardless of the thyme EO content. Likewise, they reduced the severity of grey mould caused by *B. cinerea* infection in apple. The addition of EO did not exert an antifungal effect in the fruit despite its proven antifungal action in previous *in vitro* tests. Therefore, the particular characteristics of the fruit and the interactions in the infection site (peel wounds) seriously affected the *in vivo* effectiveness of coatings of certain composition. No positive effect of lecithin was observed on the controlled postharvest parameters affecting fruit quality and physiological behaviour in either apples or persimmons; EO did not exert additional antifungal action and seemed to exert a negative effect on some other fruit quality attributes. Then, starch-gellan coatings without lecithin or thyme EO demonstrated the potential to be used in persimmons in order to control weight loss and reduce the incidence of infections caused by *A. alternata*.

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IV. GENERAL DISCUSSION

There is considerable growing interest in the development of new biodegradable packaging/coating materials obtained from renewable, natural sources, such as starch from different sources, for food applications. Starch-based edible films and coatings are an environmentally-friendly alternative to synthetic polymers due to their availability, low cost, biodegradability and food contact ability. Likewise, starch can be used in combination with other polymers or compounds to improve the functional properties of the polymeric matrix, which can also carry active compounds to better control the microbial or oxidative food decay. Edible coatings can be applied to fruits and vegetables to extend the product shelf-life through the control of water vapour and gas exchanges; thus decreasing the water loss and modifying the internal atmosphere. Despite the potential benefits gained from using edible coatings for the purposes of extending the product shelf-life and enhancing the quality and microbial safety of fresh or minimally-processed fruits and vegetables, commercial applications are still very limited. The use of starch-based materials is restricted by its high water sensitivity, which negatively affects barrier and tensile properties when compared with the conventionally-used coating materials. Different approaches (the use of plasticizers and lipids, or blending with other biopolymers, such as various gums) aim to improve these inconveniences.

The new restrictive regulations regarding fungicide residues, the reduction in the legally acceptable limits of specific fungicides and increasing public concern towards these compounds, have led to a global increase in the need to seek safer postharvest alternatives to control the decay of fruits and vegetables. The addition of essential oils (EO), with proven antifungal activity, to edible coatings based on polymeric matrices could render them more effective at prolonging the postharvest life of horticultural produce. However, in spite of the potential of EOs, the main limitation to their application for decay control is the possible induction of a strong odour or flavour in fruit or phytotoxicity risks. Encapsulation techniques could help to improve the stability of essential oils, slowing down the diffusion rate of the antimicrobial agent and maintaining a higher concentration of the active compound on the fruit surface for a longer period.

In the present Doctoral Thesis, different strategies to improve starch formulations have been analysed, in order to obtain coatings for postharvest fruit preservation. Research was focused on the selection of active compounds to target specific pathogenic fungi, subsequently studying the effect of these compounds on the film functional properties, coating spreadability on the fruit surface, and antifungal activity (both *in vitro* and *in vivo*).

The first approach, presented in **Chapter 1**, consisted of the starch's partial substitution by three microbial gums (xanthan, gellan and pullulan) in order to improve the starch film's functional properties. Thus, starch-gum blend films were obtained by casting, using glycerol as plasticiser. As regards the barrier properties, starch-gellan films exhibited the lowest WVP values in both 53 and 75% RH conditions, suggesting that the interactions between the negatively charged gellan chains and the linear amylose chains, through the formation of hydrogen bonds, could be effective at reducing the possibilities of water molecules interacting with the blend's matrix. At 53-100% RH, pure pullulan films had the highest WVP of all the formulations. The incorporation of xanthan and gellan gums contributed to a decrease in the

oxygen permeability of the films, although all the films had notably low OP values. In terms of tensile properties, in films conditioned for 1 week, the addition of the gums to the starch matrix provoked a strengthening effect reflected in the higher values of the EM compared to the net starch film, except for starch-pullulan blends conditioned at the highest RH. The film's resistance to break (TS) was considerably increased in xanthan and gellan blends, but the extensibility was generally reduced in all blend films with respect to net starch samples. In terms of ageing, blending starch with these gums inhibited starch retrogradation, since all the blend films exhibited a decrease in EM and TS values after five storage weeks. The incorporation of gellan gum led to a decrease in the water sorption capacity of the starch films, which reduced their water sensitivity. Based on these results, starch substitution in the films by gellan gum at 10 and 20 wt% was the one that most improved the barrier and tensile properties of the films, and was selected to develop starch-gellan films/coatings for the subsequent studies in the Thesis.

In **Chapter 2**, the selected starch-gellan (ratios of 9:1 and 8:2) blend formulations, were used to develop films with antifungal activity. As already commented on, the incorporation of EOs as active compounds into cast films involves heavy losses of the volatile compounds during film preparation, mainly during the film drying step. This occurrence is associated with the destabilization mechanism of the EO emulsion (droplet flocculation, coalescence and creaming) and the steam drag effect on the film surface during the solvent (water) evaporation. Then, strategies focused on promoting the emulsion stability can reduce the losses of actives. In this sense, thyme EO (0.25 or 0.5 g/g polymer) was not only incorporated by direct emulsification, but also by encapsulation in lecithin liposomes. The structural and functional properties, and the *in vitro* antifungal capacity of the resulting films, were analysed, as well as the retention of active EO in the different formulations. Lecithin encapsulation allows for the enhancement of EO retention in the films (45-55 % retention, compared to 10-20% in directly emulsified systems). Likewise, the presence of both EO and lecithin greatly modifies the films' microstructure, introducing discontinuities into the matrix and affecting their functional properties. The elastic modulus of the films was always reduced by lipid incorporation, in line with the reduction in the cohesion forces of the matrix provoked by the dispersed phase. **Figure 1** shows the map of tensile strength and percentage of elongation at break for the different film formulations, summarising the effects on these properties. All of the films with the highest proportion of gellan (8:2), with or without lipids, were less extensible than the 9:1 films, with the exception of the films with the lowest proportion of emulsified EO, which were the most extensible. In general, for a given matrix, a greater proportion of lipid (L or EO) led to a greater decrease in the fracture tension and extensibility, although this effect was dependent on the S:G ratio.

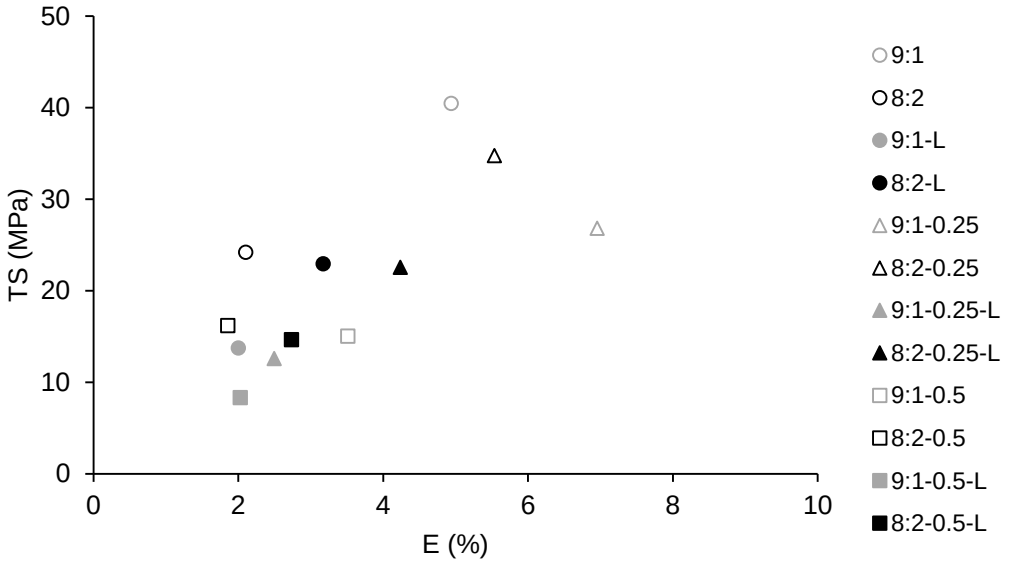

Figure 1. Map of tensile properties (tensile strength (TS) *vs.* percentage of elongation (E)) of the different film formulations with different starch:gellan ratios (grey: 9:1, black: 8:2) containing EO (0.25 or 0.5 g EO/g polymer: triangles and squares, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols) and without EO (control: circles).

As concerns barrier properties, **Figure 2** shows the map of the barrier properties for the different film formulations. Films with the lowest ratio of lecithin-encapsulated EO exhibited the lowest values of water vapour permeability (WVP), regardless of the polymer matrix, followed by the other formulations with L (with and without EO). Therefore, the presence of L and the subsequent formation of the layered structure in the film (Chapter 2) reduced the water vapour transfer rate, mainly due to the resistance offered by the lipid microlayers in the film. In contrast, the incorporation of both L and EO promoted oxygen permeability (OP) due to the hydrophobic nature of lipids, which facilitates oxygen solubility and transfer. This effect was more marked in the starch-rich matrix with L, although all of the films exhibited very low values of OP, as has been observed for starch-based films.

The optical properties of starch-gellan films were also affected by lecithin incorporation due to the natural colour of this component. Films containing lecithin were slightly darker (change in L* from 77 to about 72), exhibiting a more saturated colour with a yellowish hue. **Figure 3** shows the a*-b* chromatic plane with the chromatic *locus* of the different films, where the two different groups (with and without lecithin films) can be observed. Nevertheless, given the thinness of the films, these changes have no real relevance in practical applications.

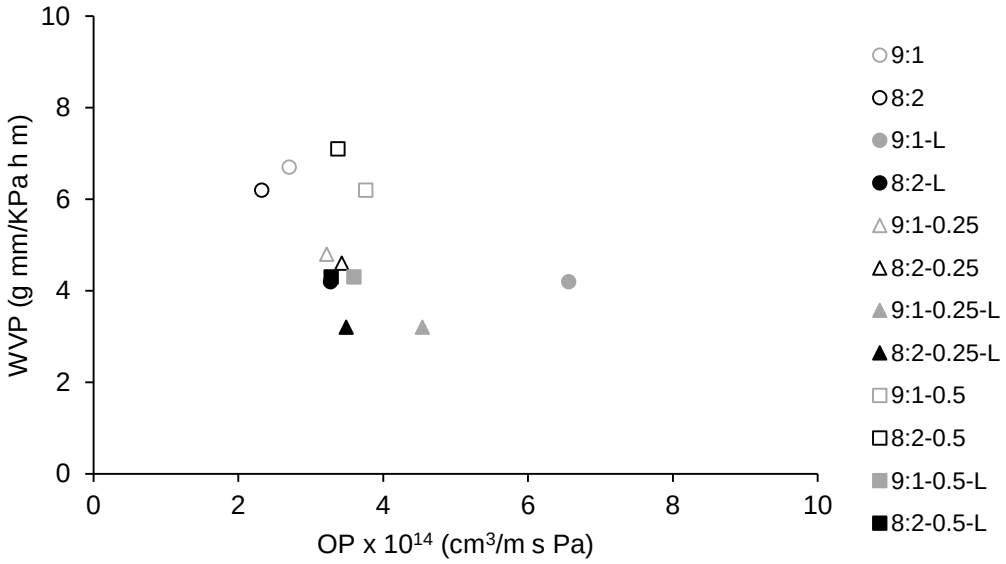

Figure 2. Map of barrier properties (water vapour permeability (WVP) *vs.* oxygen permeability (OP)) of the different film formulations with different starch:gellan ratios (grey: 9:1, black: 8:2) containing EO (0.25 or 0.5 g EO/g polymer: triangles and squares, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols) and without EO (control: circles).

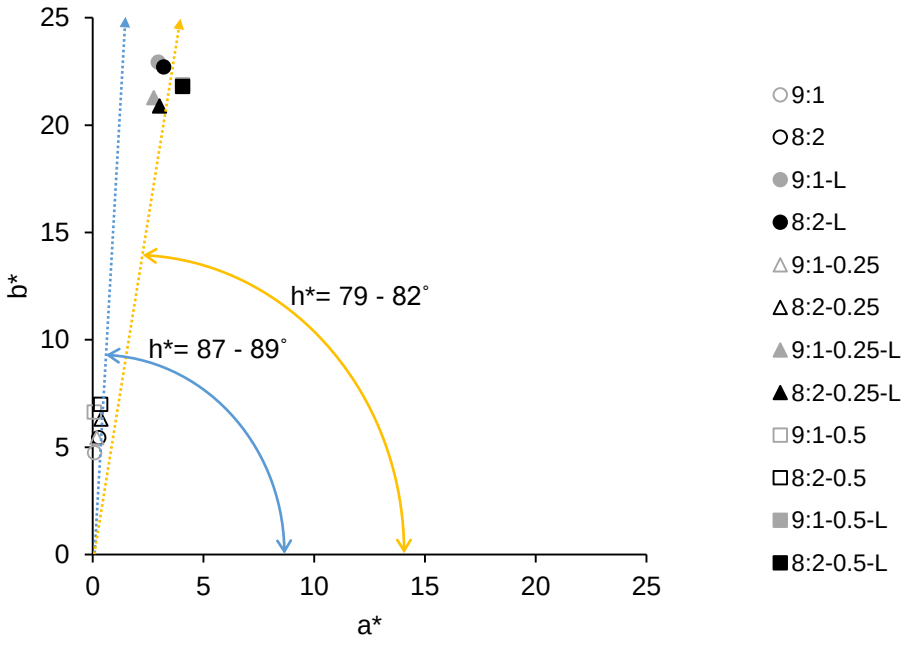

Figure 3. Chromatic map (a**vs.* b*) of the different film formulations with different starch:gellan ratios (grey: 9:1, black: 8:2) containing EO (0.25 or 0.5 g EO/g polymer: triangles and squares, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols) and without EO (control: circles). Range of hue (h*) values are indicated for each group of films.

As concerns the *in vitro* antifungal action, **Figure 4** summarizes the results of radial growth after 7 days of *A. alternata* and *B. cinerea* when the different films (with and without EO) were applied on the culture plate. All of the films containing EO inhibited the radial growth of the fungi, according to the final content in the film. However, thyme EO was more effective against *B. cinerea* than *A. alternata*. In fact, beyond a certain EO content in the films, no growth of *B. cinerea* was observed throughout the tested period, thus indicating a total fungicide action. In both fungi, an effect of the film's matrix composition was observed both in the control samples and in active films, where a higher content of starch enhanced fungal growth, limiting the action of EO. At the highest EO content, the effect of the matrix was less remarkable, suggesting that the films require relatively high contents of EO to ensure a good antifungal action. These sufficiently high EO concentrations were only reached when lecithin encapsulation was used to prevent losses of the EO during the film drying step. In the case of *B. cinerea*, all of the films with the highest encapsulated EO content completely inhibited the growth of the fungus. On the basis of the obtained results, S:G blend formulations at 8:2 ratio were used for fruit applications.

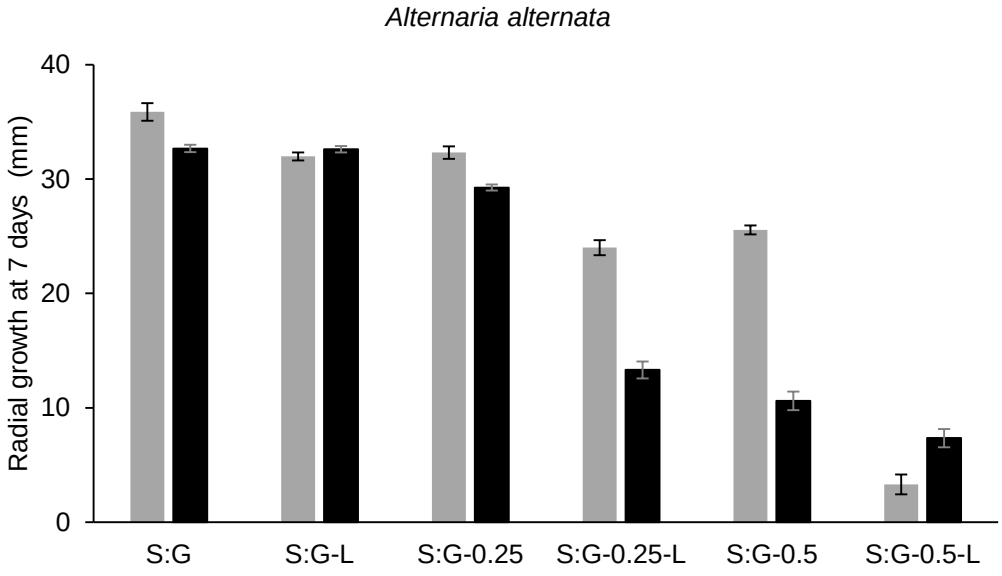

Figure 4. Radial growth of *Alternaria alternata* and *Botrytis cinerea* after 7 days for each film formulation with different starch:gellan ratios (grey: 9:1, black: 8:2) containing EO (0.25 or 0.5 g EO/g polymer), emulsified or lecithin-encapsulated and without EO (control).

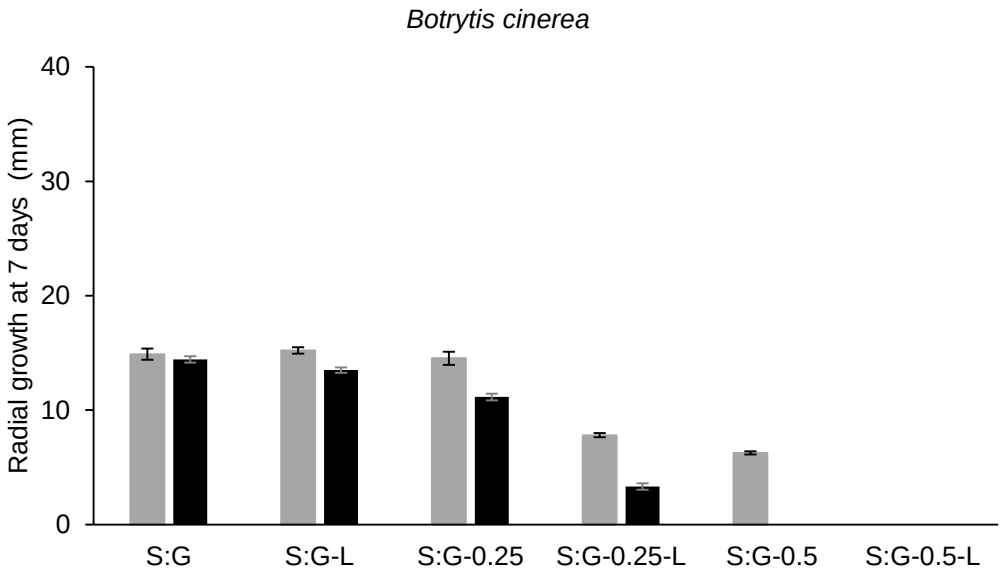

Figure 4. (Continued).

An important issue related with the effectiveness of edible coatings at preserving fruits and vegetables is the product wettability with the film forming dispersion to obtain a uniformly coated surface. This is influenced both by the fruit/vegetable surface properties and by the chemical composition and molecular interactions of the coating-forming solution/dispersion. Prior to applying the starch-gellan (8:2) formulations as fruit coatings, in **Chapter 3**, the surface properties of apple, tomato and persimmon were analysed as well as the spreadability of the liquid formulations on the fruit surface. Different concentrations of Tween 85 were incorporated into the starch-gellan coating-forming systems (with and without EO) in order to explore its potentially beneficial effect on the coating spreadability. The different fruit skins behaved as low-energy surfaces. Tween 85 positively influenced the values of the contact angles and surface tension of EO-free formulations. However, in the presence of emulsified or lecithin-encapsulated thyme EO, the surfactant exerted a negative effect, depending on its concentration. The wettability of EO-free formulations was notably improved with Tween 85 at $5 \cdot 10^4$ mg/L, whereas coating-forming systems containing emulsified or encapsulated EO did not require surfactant to improve their already good spreadability. The requirement of such a high Tween 85 concentration was attributed to the formation of amylose-surfactant complexes that limit the surface activity of the compound.

Finally, in **Chapter 4,** starch-gellan (8:2) coating formulations with thyme essential oil were applied to apples and persimmons to analyse their effectiveness at controlling the postharvest behaviour of coated fruit and fungal decay. According to the results obtained in **Chapter 3**, Tween 85 was also added to the S:G formulation and tested in a preliminary trial on apples, in comparison with the formulation without Tween 85. However, the addition of Tween 85 to the S:G formulation was discarded on the basis of the observed negative effects on the fruit weight loss and texture. The interactions of surfactant at such a high concentration with the natural wax of fruit could change the barrier properties of the wax-coating assembly.

The following figures summarize the main results obtained for respiration rates, weight loss, and fruit texture after 14 storage days of samples coated with the different formulations, in comparison with non-coated fruit.

Coatings had a different effect on respiration rates depending on whether the fruit was apple or persimmon, which could be attributed to the different physiological patterns of the fruits and the specific interactions with the coatings. Although the coating was not observed to have a relevant effect on the respiration rates of persimmons, taking the variability into account, the respiration rates and quotient in apples were promoted. In this specific case, coatings tend to increase the O_2 consumption and CO_2 production rates with respect to the control sample, the latter being more enhanced. Then, especially in apples, coatings tend to shift respiration to a more anaerobic pattern. **Figure 5** shows the map of respiration rates for the studied fruit, where these effects can be observed.

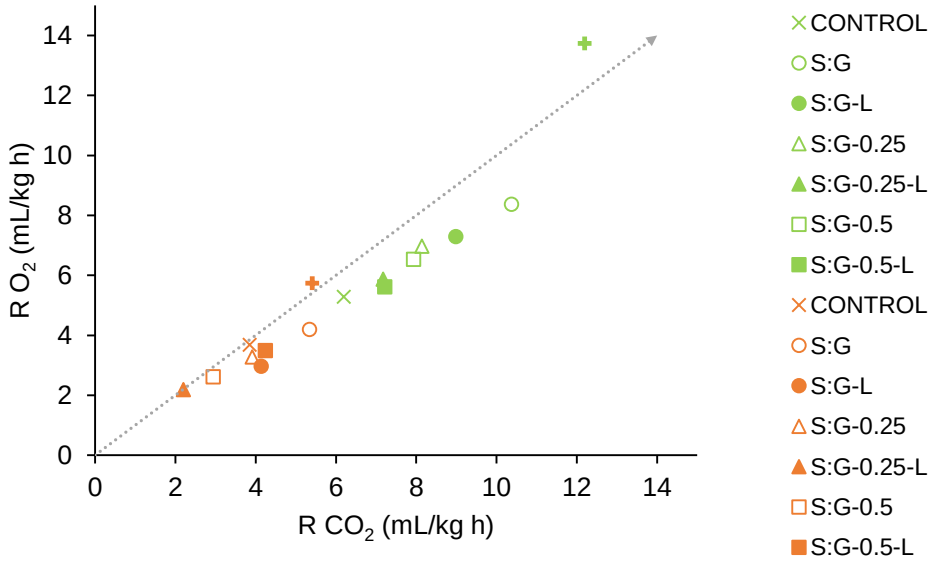

Figure 5. Map of the respiration rates (consumption of O_2 *vs.* production of CO_2) of coated and uncoated 'Golden Delicious' apples (green) and 'Rojo Brillante' persimmons (orange) for 14 days of storage at 25 ˚C. Coatings with different EO concentrations (0.25 or 0.5 g EO/g polymer: triangles and squares, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols) and without EO (circles). Uncoated fruit were used as control at this time (x) and at initial time (+). Line represents the *locus* for RQ=1.

Figure 6 shows a map for the values of the F_{max} and d_{max} of the different coated and uncoated fruits after 14 days of storage. No significant changes in the maximum puncture force (failure point) were observed for either uncoated or coated apples after storage with respect to the initial values before storage. The maximum penetration distance was slightly longer than before storage, which can be associated with a loss of cellular turgidity due to the superficial dehydration of the apples. However, no significant differences were observed between coated and control apples at the end of the storage. In persimmons, the coatings had the effect of

maintaining the firmness of the fruit (F_{max}), but the values of the maximum penetration distance at the failure point after storage were higher for most of the coated samples than for the uncoated. These textural changes could be related with the progress in maturity and water loss of the tissue over time. The increase in F_{max} values must be attributed to the greater deformability of the tissue, allowing for deeper penetration without failure, thus accumulating more compression and shear resistance. The smallest changes occurred in the sample coated with the formulation containing lecithin without EO.

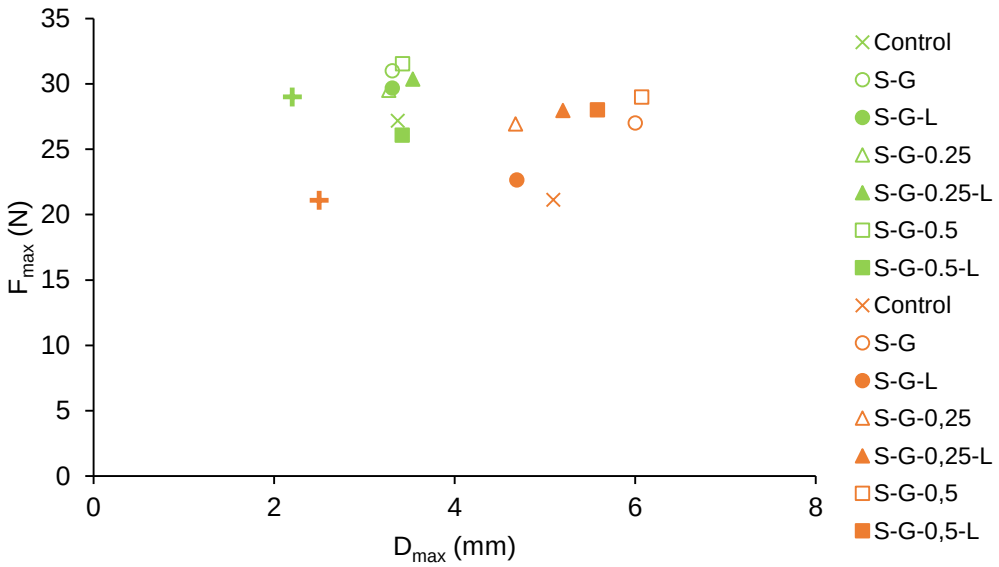

Figure 6. Map of the maximum puncture force (F_{max}) *vs.* penetration distance (d_{max}) of coated and uncoated 'Golden Delicious' apples (green) and 'Rojo Brillante' persimmons (orange) for 14 days of storage at 25 ˚C. Coatings with different EO concentrations (0.25 or 0.5 g EO/g polymer: triangles and squares, respectively), emulsified (empty symbols) or lecithin-encapsulated (full symbols) and without EO (circles). Uncoated fruit were used as control at this time (x) and at initial time (+).

As concerns water loss (**Figure 7**), the rate of relative weight loss after 14 days of storage was not significantly affected by coating application or composition (between 0.20-0.23 $days^{-1}$) in apples. Water loss rates were higher in persimmons than in apples and slightly varied depending on the coating formulation. The highest values were obtained for both uncoated samples and those coated with the formulation containing the highest content of lecithin-encapsulated EO (maximum lipid content in the film) and the lowest value (0.52 day-1) was obtained for the samples coated with the formulation with emulsified EO (without lecithin) in the lowest ratio (minimum lipid content in the film). Based on these results, persimmon fruits seem to be more sensitive to dehydration than apples under these storage conditions and the coatings with the lowest ratio of emulsified EO exerted a protective effect. None of the coatings reduced weight loss in apples and only the coating that had the lowest amount of EO and no lecithin prevented water loss in persimmons. Hydrophilic coatings are usually not so effective

at controlling the weight loss of fruit, but lipid incorporation can mitigate this drawback. However the lipids used in this study did not notably improve the effectiveness of the coatings in this sense, which could be related with the lipid interactions with the fruit cuticle that affect the overall barrier capacity of the wax-coating assembly. In fact, although isolated films exhibited reduced WVP when they contained lecithin or OE, this reduction was not effective when a thin coating was applied on the fruit surface.

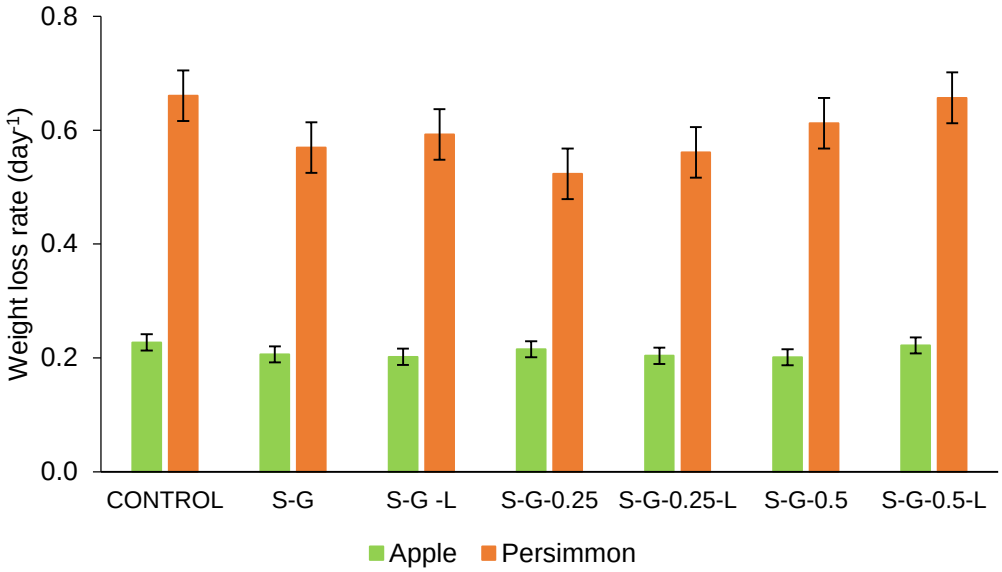

Figure 7. Weight loss rate (day^{-1}) of coated and uncoated 'Golden Delicious' apples and 'Rojo Brillante' persimmons for 14 days of storage at 25 ˚C.

Finally, **Figure 8** summarizes the results obtained in the *in vivo* antifungal test on apple (grey mould) and persimmon (black spot) after 12 days of incubation of the inoculated fruit. The applications of starch-gellan coatings did not significantly reduce the disease incidence on apples inoculated with *B. cinerea*, as compared to non-coated ones (100 ± 0 % of incidence). Despite what was observed in the *in vitro* study, where starch-gellan films with thyme EO exhibited a marked antifungal effect, when applied on fruit, no effect of the addition of EO was observed. However, all coatings, regardless of their composition, significantly reduced the severity of grey mould with respect to the control samples (100 mm of lesion diameter), with no particular effect of the EO. Starch-gellan coatings were more effective at reducing the incidence of black spot caused by *A. alternata* on persimmon (73 ± 5 % of incidence in non-coated ones), although coatings containing lecithin were not effective and the presence of EO had no significant effect. Disease severity was significantly reduced in coated persimmons, when compared to the control samples (21.9 ± 1.9 mm of lesion diameter) after 12 days of incubation, regardless of the coating composition.

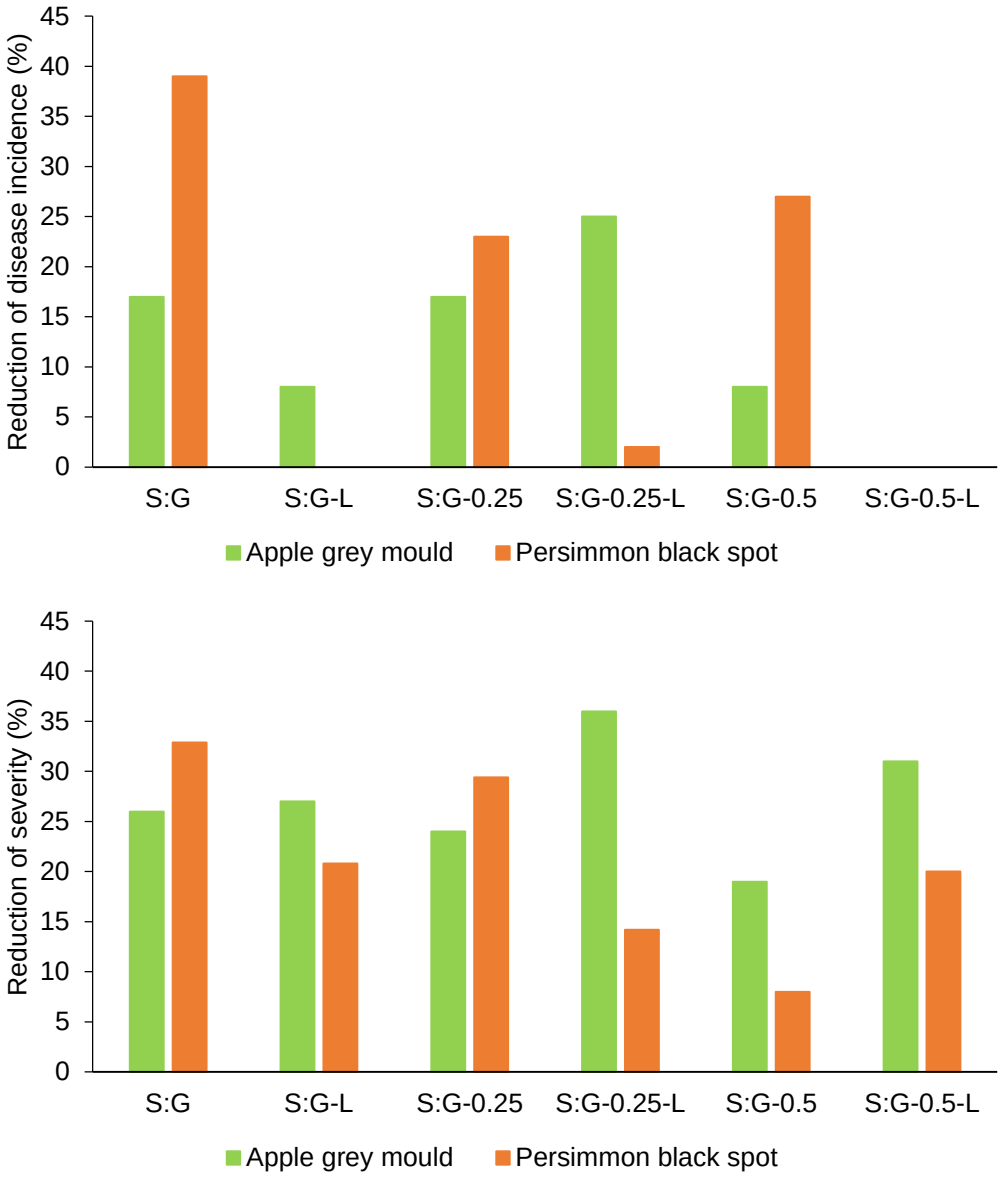

Figure 8. Mean values of the reduction (with respect to the uncoated sample) in disease incidence and severity of grey mould on apples artificially inoculated with *B. cinerea* and black spot on persimmons artificially inoculated with *A. alternata*, after 12 days of incubation.

The main conclusion is that the incorporation of thyme essential oil into starch-gellan coatings did not improve their functionality when applied on apple and persimmon as regards the fruit preservation capacity: neither the respiration, nor the weight loss, nor the texture nor fungal decay. Then, formulations without EO could be applied to prevent weight loss or black spot in persimmon, although they were less effective in apples. So, the *in vivo* effectiveness of EOs cannot be anticipated by their antifungal activity in *in vitro* tests and the interactions between EOs and fungal pathogens are modulated by the fruit host and the conditions in the infection court. Therefore, the interactions of coatings and their components with the fruit always constitute a distinguishing factor to define the particular behaviour of coated fruit and, in the

case of coatings formulated with antifungal ingredients, these interactions can affect the *in vivo* disease control ability of the coating. Numerous factors, such as the nature of the coating components, the type and concentration of the antifungal compound(s), the species and strain of the target postharvest pathogen, the species, cultivar and physical and physiological condition of the fruit host and the postharvest environmental conditions, can influence the antifungal and other functional properties of the coatings.

V. CONCLUSIONS

1. Blending starch with gellan gum was effective at reducing the moisture sorption capacity of starch films and also reduced the water vapour and oxygen permeability and increased the film strength and resistance to break, without markedly limiting the film stretchability. Likewise, gellan-starch blends preserve films against the phenomenon of retrogradation. Xanthan gum was effective at increasing the tensile strength of the starch films but did not reduce their water sorption capacity and water vapour permeability. Pullulan was not so effective at enhancing the barrier capacity of starch films or increasing tensile strength. From the obtained results, starch substitution in the films by gellan gum at both 10 and 20 wt % is the one that most improved the barrier and tensile properties of the films and can be used to obtain films that are more useful for food packaging purposes. Particularly, foods such as nuts or snacks with low oxygen pressure requirements to avoid oxidations, and without free water to avoid film moisturising, may be successfully packaged, allowing to extend their self-life.

2. Starch-gellan blend films containing thyme essential oil (EO) exhibited antifungal effect in in vitro tests against *A. alternata* (AA) and *B. cinerea* (BC), the second being more sensitive to the action of the EO. The antifungal action was correlated with the residual content of the oil in the film after the drying step and was slightly affected by the polymer matrix composition (9:1 or 8:2 S:G ratio). A greater amount of starch in the film protected the fungi, making their growth faster, when the active content was relatively low. The growth of AA was greatly inhibited when the EO content exceeded 0.05 g/g film, whereas BC was completely inhibited when films contained more than 0.053 g EO/ g film. Lecithin encapsulation of the EO greatly contributed to the EO retention in the film during film formation, which enhanced the film's antifungal action. Therefore, lecithin enhanced the film's water barrier properties, whereas all of the films exhibited high oxygen barrier capacity. Lecithin imparted a slightly brownish color to the films, but improved their gloss while reducing film stiffness and resistance to break and extensibility. Then, films with lecithin-encapsulated EO, with a S:G ratio of 8:2 were very effective at controlling fungal growth, while exhibiting adequate functional properties as packaging/coating materials.

3. The skins of apple, tomato and persimmon were found to be of low-energy surfaces, their surface tension and critical surface tension values being, respectively, 29.2 and 22.7 mN/m for apple, 21.7 and 16.1 mN/m for tomato, and 25.4 and 20.3 mN/m for persimmon. The polar components were 9.4, 3.6 and 6.0 mN/m, and the dispersive components 19.8, 18.2 and 19.4 mN/m, respectively, for apple, tomato and persimmon, which demonstrates the ability of these surfaces to interact with non-polar liquids. The addition of Tween 85 positively influenced the contact angle and surface tension values of the starch-gellan solutions, but in the presence of thyme essential oil and lecithin, it had a negative impact depending on the concentration of the surfactant. Ws was notably improved with Tween 85 at $5 \cdot 10^4$ mg/L in the S-G formulations (values closer to zero). However, it had a negative effect on the already good spreadability of S:G-EO and S:G-EO-L coatings. These findings provide relevant information on surface properties of starch-gellan coating-forming solutions in view of their use as coatings for fruits. However, it would be necessary to analyse the effect of the coatings with Tween 85 on the

preservation parameters of fruits, since this type of compound could also interact with the wax layer of the fruits, modifying their natural barrier capacity.

4. Starch-gellan coatings, containing or not emulsified or lecithin-encapsulated EO, applied on apples and persimmons had a different effect on the postharvest parameters (weight loss, respiration rates and firmness changes), depending on the coating composition and type of fruit. None of the coating formulations reduced the weight loss in apples, although they prevented water loss in persimmons. In contrast, although the coating was not observed to have any significant effect on the respiration rates and respiration quotient of persimmons, the respiration rates and quotient in apples were promoted. Coatings did not affect the changes in fruit firmness in apples or persimmons; nevertheless, in the latter these may be mainly associated with the ripening progress. Regarding fungal decay, coatings without lecithin reduced the incidence of black spot caused by *A. alternata* in persimmons, regardless of the thyme EO content. Likewise, they reduced the severity of grey mould caused by *B. cinerea* infection in apple. The addition of EO did not exert an antifungal effect in the fruit despite its proven antifungal action in previous in vitro tests. Therefore, the particular characteristics of the fruit and the interactions in the infection site (peel wounds) seriously affected the *in vivo* effectiveness of coatings of certain composition. No positive effect of lecithin was observed on the controlled postharvest parameters affecting fruit quality and physiological behaviour in either apples or persimmons; EO did not exert additional antifungal action and seemed to exert a negative effect on some other fruit quality attributes. Then, starch-gellan coatings without lecithin or EO demonstrated the potential to be used in persimmons in order to control weight loss and reduce the incidence of infections caused by *A. alternata*.

The **final conclusion** is that the incorporation of thyme essential oil into starch-gellan coatings did not improve their functionality when applied on apple and persimmon as regards the fruit preservation capacity: neither the respiration, nor the weight loss, nor the texture nor fungal decay. Then, formulations without EO could be applied to prevent weight loss or black spot in persimmon, although they were less effective in apples. So, the in vivo effectiveness of EOs cannot be anticipated by their antifungal activity in in vitro tests and the interactions between EOs and fungal pathogens are modulated by the fruit host and the conditions in the infection court. Therefore, the interactions of coatings and their components with the fruit always constitute a distinguishing factor to define the particular behaviour of coated fruit and, in the case of coatings formulated with antifungal ingredients, these interactions can affect the *in vivo* disease control ability of the coating. Numerous factors, such as the nature of the coating components, the type and concentration of the antifungal compound(s), the species and strain of the target postharvest pathogen, the species, cultivar and physical and physiological condition of the fruit host and the postharvest environmental conditions, can influence the antifungal and other functional properties of the coatings.