Antifungal edible coatings for postharvest preservation of fresh fruit

L. Palou[a], M.B. Pérez-Gago

Laboratori de Patologia, Centre de Tecnologia Postcollita (CTP), Institut Valencià d´Investigacions Agràries (IVIA), Montcada, València, Spain.

Abstract

Postharvest losses of fresh fruit are mainly caused by weight loss, physiological disorders, and decay during storage and commercialization. Currently, postharvest treatments with conventional chemical fungicides and/or synthetic waxes are commonly used in combination with low-temperature storage to reduce such losses and minimize their economic impact. However, their continuous use by the industry for many years has arisen important health and environmental problems related to the production of chemical residues and the proliferation of resistant pathogenic fungal biotypes. Therefore, safe and eco-friendly alternatives should be commercially implemented as part of non-polluting integrated disease management (NPIDM) programs for preservation of fresh fruit. Among them, the development of edible coatings with antifungal activity is a technological challenge and a very active research field worldwide. The main advantage of these coatings is that they could provide a single solution for both physiological and pathological major postharvest issues. While some natural coatings such as chitosan or *Aloe* spp. gels show inherent antifungal activity, specific food-grade antifungal ingredients should be incorporated into composite matrixes of hydrocolloids (polysaccharides such as cellulose derivatives, alginates, pectins, gums, and peptides or proteins) and lipids to form synthetic edible coatings with antifungal properties. These ingredients include natural or low-toxicity compounds, such as inorganic or organic salts (e.g., carbonates, sorbates, benzoates, paraben salts) and essential oils or other plant extracts approved as food additives or generally recognized as safe (GRAS) compounds by competent authorities, and biological control agents such as antagonistic strains of some microorganisms.

Keywords: fungal postharvest diseases, fungicide-free control, chitosan, composite edible coatings, GRAS compounds, biocontrol agents

INTRODUCTION

Postharvest handling of fresh fruit in commercial packinghouses is intended to commercialize fruit of maximum quality, increase their postharvest life, and reduce produce losses. In general, postharvest losses can be of physical, physiological or pathological origin. Physical losses are typically due to rind wounds or bruises caused during harvest, transportation or postharvest handling in the packinghouse. These peel injuries are not only important for causing direct losses, but also for being infection sites for economically important postharvest pathogens. Other pathological losses are caused by latent pathogens that infect flowers or young fruit in the field but develop after harvest. Postharvest physiological losses can also be originated in the orchard or caused by inappropriate handling or storage conditions in the packinghouse or during commercialization.

[a] E-mail: palou_llu@gva.es

Fruit postharvest diseases have been controlled worldwide for many years through postharvest applications of synthetic chemical fungicides. Active ingredients such as imazalil (IMZ), thiabendazole (TBZ), pyrimethanil (PYR), fludioxonil (FLU), and others are being extensively used in commercial packinghouses as cost-effective means of postharvest decay control. However, the intense and continuous use of these conventional fungicides is causing important problems, such as human health issues and environmental contamination due to chemical residues, reduced efficacy due to the proliferation of resistant fungal biotypes, and restricted access to new high-value organic markets or traditional export markets that are now demanding products with lower levels of pesticides to satisfy consumer demands (Palou et al., 2016). Therefore, consumer trends and legislative updates clearly favor a reduction in the use of conventional fungicides, which makes it necessary to potentiate research to develop and implement alternative approaches and novel technologies for the control of postharvest diseases. Effective fungicide-free control will need to adopt integrated strategies in which, besides new non-polluting postharvest antifungal treatments, all factors affecting disease epidemiology and incidence will need to be taken into account, including preharvest factors. Such a "non-polluting integrated disease management" (NPIDM) concept should not be confused with the traditional "integrated disease management" (IDM) in the context of agricultural "integrated production," which often implies fruit production in compliance with particular national or regional regulations and programs that still include the use of postharvest fungicides (Palou, 2018). The establishment of NPIDM strategies is based on the comprehensive knowledge of pathogen biology and epidemiology and needs to consider all preharvest, harvest, and postharvest factors that can influence the incidence of postharvest diseases in order to minimize economic losses. All the actions planned, in the field or after harvest, should be cost-effective and not adversely affect fruit quality. Actions in the field will be especially important in the case of postharvest diseases caused by latent pathogens. In any case, however, the basis of successful NPIDM strategies is the commercial adoption of suitable non-polluting postharvest antifungal treatments to replace the use of conventional fungicides. In general, according to their nature, these alternative treatments can be physical, chemical, or biological (Wisniewski et al., 2016). Chemical alternatives should be compounds with known and minimal toxicological effects on mammals and impact on the environment and, among them, antifungal edible coatings have increasingly arisen interest among researchers and manufacturers as a promising novel technology not only intended to reduce decay losses, but also to confront postharvest physiological problems, thus extending considerably the postharvest life of fresh fruit (Palou et al., 2015).

Postharvest fruit coating is a common practice to replace the natural waxes that can be removed during fruit washing and handling in the packingline. Commercial coatings are generically known as waxes and they are anionic microemulsions containing resins and/or waxes such as shellac, carnauba wax, beeswax, polyethylene, or petroleum waxes. Their main purpose is to reduce fruit weight loss, respiration, shrinkage and improve appearance, but they can also reduce the incidence of physiological rind disorders such as chilling injury and, if amended with chemical fungicides, control postharvest diseases. Edible coatings are biodegradable formulations intended to replace these currently used commercial waxes, thus avoiding the use of synthetic components such as polyethylene wax, ammonia, and morpholine. The concept of antimicrobial edible coatings emerges when edible coatings also present inherent antimicrobial activity and if this activity is specifically antifungal, we then refer to them as antifungal edible coatings (Palou et al., 2015; Pérez-Gago and Palou, 2016). Antifungal edible coatings can be natural if they are directly obtained from plants, animals, or microorganisms or synthetic if they are manufactured through the combination of different edible ingredients. The antifungal properties of the latter are typically provided by the addition of food-grade antifungal compounds to biodegradable coating matrixes.

[a] E-mail: palou_llu@gva.es

NATURAL ANTIFUNGAL EDIBLE COATINGS

Chitosan-based coatings

Chitosan is a natural biopolymer with antimicrobial activity that has the property to form edible coatings. It is a linear cationic polysaccharide of high molecular weight consisting of 1,4-linked 2-amino-deoxy-β-D-glucan, a partially deacetylated derivative from chitin. Chitin is present in the exoskeletons of crustaceans (crabs, lobsters, shrimps, etc.) and is, after cellulose, the most abundant polysaccharide in nature (Romanazzi et al., 2018). Chitosan is produced commercially with different deacetylated grades and molecular weights, which are related to their functional properties and antifungal effects. Chitosan hydrochloride was approved in the European Union (EU) as the first product in the list of basic substances in plant disease management (Regulation EU 563/2004) and some commercial formulations are registered for use as plant protection products.

Chitosan and derivatives are currently the most assayed antifungal edible coatings for postharvest preservation of fresh fruit. They have been investigated either alone or formulated with other additional antifungal ingredients. Their beneficial effect for postharvest disease reduction has been reported for a wide variety of fruits including citrus, apples, mango, grapes, strawberries, small berries, or tomatoes (Romanazzi et al., 2017; Oliveira et al., 2018). Its antimicrobial activity, however, depends on several factors such as the type of chitosan, degree of acetylation, molecular weight, concentration, medium pH, target microorganism, and presence of other ingredients in the chitosan coating matrix. In general, chitosan-amended coatings allow a gradual release of the added antifungal and provide additional properties for fungal growth inhibition and fruit quality maintenance (Palou et al., 2016). The most important of these additional antifungal ingredients are essential oils (El-Mohamedy et al., 2015; de Oliveira et al., 2017), although others such as biocontrol agents have also been assayed (El-Ghaouth et al., 2000). The activity of chitosan has also been improved by the development of bilayer coatings comprised of chitosan and another natural polymer such as carboxymethyl cellulose (CMC) (Arnon et al., 2014). Nanotechnology has also been applied for development of innovative chitosan-based coatings. Chitosan nanoformulations allowed for encapsulation of functional ingredients and reduced chemical degradation, which resulted in increased antifungal activity (Ali et al., 2013; Mustafa et al., 2013).

Aloe spp. gels

Gels from the leaves of the plant *Aloe vera*, but also from other *Aloe* spp. such as *A. arborescens* or *A. ferox*, show well-known bioactive and antimicrobial activity and have been used as raw materials for many uses including the postharvest treatment of fresh fruit (Zapata et al., 2013; Ortega-Toro et al., 2017). Similar to chitosan, the physical and chemical properties of the gels allow their use as edible coatings with physiological and pathological functionalities. Different research works showed both in vitro and in vivo inhibitory activity of *A. vera* gels against important postharvest pathogens of stone fruits, table grapes, strawberries, or avocados (Martínez-Romero et al., 2006; Bill et al., 2014; Sogvar et al., 2016). Most of these studies also demonstrated the ability of these coatings to delay ripening and preserve functional properties and overall quality of treated fruit. Furthermore, the activity of *Aloe* spp. coatings has been recently reinforced in some cases through the incorporation of additional ingredients such as essentials oils, GRAS acids or salts, chitosan, and biocontrol yeasts (Bill et al., 2014; Sogvar et al., 2016; Vieira et al., 2016; Martínez-Romero et al., 2017; Jiwanit et al., 2018).

SYNTHETIC ANTIFUNGAL EDIBLE COATINGS

[a] E-mail: palou_llu@gva.es

Coating matrixes

In general, coating matrixes for fruits and vegetables are polysaccharides, proteins, lipids, or a mixture of them (composite coatings, mainly by combination of hydrocolloids and lipids). Common polysaccharides studied for the development of synthetic edible coatings for fruits are starch and derivatives, cellulose and derivatives, pectin, alginate, carrageenan, and some gums. In the case of proteins, those that have received more attention as coatings for fruits include casein, whey, soy, zein, gluten, and collagen; although some works can be also found with proteins of limited availability derived from cottonseed, peanut, rice, pea, pistachio grain sorghum, or egg albumen proteins, among others. Lipid compounds commonly used in edible coatings include plant and animal natural waxes such as beeswax, candelilla, carnauba, rice bran; animal and vegetal native oils and fats such as peanut, coconut, olive, lard; and fractionated, concentrated, hydrogenated and/or reconstituted oils and fats such as fatty acids, mono-, di-, and triglycerides, cocoa butter, milk fat fraction, margarine, shortenings, among others (Pérez-Gago and Palou, 2016).

These main ingredients present advantages and disadvantages when used as fruit coatings. Generally, lipids offer a good moisture barrier due to their hydrophobic nature, reducing water loss, shriveling, and shrinkage of coated fruit. However, their non-polymeric nature limits their ability to form cohesive films with good integrity. On the contrary, proteins and polysaccharides are good film-formers, given by their polymeric structure, and present a good oxygen barrier at medium-high relative humidity (RH), which helps controlling the gas exchange between the fruit and the environment. However, their hydrophilic nature makes them poor moisture barriers. For this reason, most edible coatings for fruits and vegetables contain a combination of one or several hydrocolloids as hydrophilic components, to provide the structural and gas barrier, and lipids as hydrophobic components, to provide the moisture barrier, forming composite coatings (Valencia-Chamorro et al., 2011).

Beside these main ingredients that constitute the coating matrix and the antifungal ingredients that will be described in the next section, several other compounds may be added in the formulations to improve coating performance (e.g., plasticizers and emulsifiers) or provide additional functionalities (e.g., texture enhancers, antioxidants, nutraceuticals, and nutrients). Among them, plasticizers (e.g., sucrose, glycerol, sorbitol, propylene glycol, polyethylene glycol, fatty acids, and monoglycerides) and emulsifiers (e.g., fatty acids, ethylene glycol monostearate, glycerol monostearate, esters of fatty acids, lecithin, sucrose ester, and sorbitan monostearate or polysorbates (tweens)) are key minor ingredients in composite edible coating formulations that improve coating integrity and emulsion stability, respectively, by decreasing the intermolecular forces between polymer chains and reducing surface tension between the hydrophilic/hydrophobic phases (Han, 2014). Furthermore, in many cases, they are also used to ensure good surface wetting, spreading, and adhesion of the coating to the fruit surface (Sapper et al., 2019). The effect of these key minor ingredients in the barrier and mechanical properties of stand-alone films have been extensively studied in different hydrocolloid matrixes and some reviews can be found in the literature (Dhumal and Sarkar, 2018; Hassan et al., 2018).

Antifungal ingredients

The antifungal agents that can be used as functional ingredients of edible coatings to control postharvest fungal decay include permitted synthetic food additives and generally recognized as safe (GRAS) compounds [status affirmed by the European Food Safety Authority (EFSA), the United States Food and Drug Administration (US FDA), or equivalent national legislations of other countries]; natural antimicrobial compounds from plant or animal origin; other low-toxicity chemicals approved for specific uses and conditions that pose minimal risk of damage to both consumer's health and environment, such as metal-

[a] E-mail: palou_llu@gva.es

based nanoparticles or nanocomposites; and microbial antagonists that perform as biological control agents (Palou et al., 2016). Each antifungal ingredient has specific inhibitory mechanisms and activity against different pathogenic microorganisms that mainly depends on their chemical/biological composition/activity (Pérez-Gago and Palou, 2016). Table 1 shows antifungal agents within the different groups that could be incorporated into edible films and coatings.

Table 1. Examples of antifungal agents that could be used as ingredients of edible films and coatings.

Food additives and GRAS salts • *Organic acids*: Acetic, benzoic, citric, lactic, malic, propionic, sorbic, tartaric • *Organic salts*: Sodium acetate, sodium diacetate, sodium benzoate, sodium citrate, sodium formate, calcium formate, sodium L-lactate, sodium propionate, calcium propionate, potassium sorbate, sodium L-tartrate • *Inorganic salts:* sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, ammonium carbonate, ammonium bicarbonate, potassium silicate, sodium silicate • *Parabens*: Methylparaben, ethylparaben, sodium salt of methylparaben, sodium salt of ethylparaben
Natural compounds • *Essential oils and spices:* Cinnamon, lemongrass, oregano, carvacrol, cinnamaldehyde, citral, capsicum, thymol • *Plant extracts*: Grape seed extracts, pomegranate peel extracts, avocado seed extracts, rosemary extracts, garlic extracts, vanilla, vanillin
Antifungal proteins and peptides Lysozyme, peroxidase, lactoperoxidase, lactoferrin, natamycin
Metal-based nanoparticles Ag, ZnO, TiO_2, SiO_2, Al_2O_3, Fe_3O_4, Fe_2O_3
Biocontrol agents • Bacteria, yeast, yeast-like fungi, and filamentous fungi: *Candida sake, C. laurentii, Lactobacillus plantarum, Pantoea agglomerans, Wickerhamomyces anomalus, Cryptococcus laurentii, Bacillus subtilis*

Among food additives and GRAS compounds, organic and inorganic acids and their salts are the most important because of their great availability, ease of handling and use, and low cost. Salts are preferred due to their higher solubility in water (Palou et al., 2016). These compounds are non-specific antimicrobials with the advantage that GRAS materials are exempt from residue tolerances on all agricultural commodities by the US FDA. Their optimal inhibitory activity occurs at low pH, when the microorganism cell membranes are uncharged and the dissociated form of the acids can freely diffuse across the cell membrane, resulting in cytoplasm acidification. Thus, for example, optimum pH values for propionate or benzoate salts are 5.0-5.5 and 4.0-4.5, respectively, although others such as sorbates may be also effective at pH values as high as 7.0 (Valencia-Chamorro et al., 2011).

Parabens are the alkyl esters of para-hydroxybenzoic acid and present inhibitory activity against fungi causing fruit postharvest decay (Moscoso-Ramírez et al., 2013) and also against other microorganisms such as bacteria. Their antimicrobial activity relies on the propenoid side chain that facilitates their transport across the cell membrane. Therefore, their inhibitory activity generally increases as the alkyl chain length of parabens increases (Corrales et al., 2014). The optimum pH for effective antimicrobial activity of parabens is in the range 3.0-8.0 (Valencia-Chamorro et al., 2011). In the EU, methyl-, and ethyl- parabens and their respective sodium salts are currently permitted as food additives with an acceptable daily intake from 0 to 10 mg kg^{-1} (Directive EU 1995/2/EC). Propylparaben and

[a] E-mail: palou_llu@gva.es

sodium propylparaben, however, were excluded from the EU list of food additives (EU CR, 2011).

Antifungal peptides and proteins are typically short compounds of amphipathic cationic nature, which mechanism of action is presumably the disruption of the target fungal cell membrane. Among the natural polypeptides with antimicrobial activity, enzymes such as lysozyme, glucose oxidase, and lactoperoxidase present different inhibitory activity against important fungi. In spite of the interest and potential of enzymes as antimicrobials, only few of them have been authorized as food additives in the EU, including lysozyme. Natamycin, a tetraene polyene macrolide, is a natural antifungal agent produced by the bacterium *Streptomyces natalensis*. It is very active against nearly all molds and yeasts. In the EU, it is not of safety concern for food applications (EFSA, 2009), and the US FDA classifies it as a GRAS compound (Corrales et al., 2014). Other natural peptides with activity against postharvest fungi are those produced by several biological control agents, mainly bacteria, such as iturins and fengycins (Palou et al., 2016). Limitations of natural peptides such as non-specific toxicity, low stability, and poor bioavailability have led to attempts to artificially synthetize new antifungal peptides with superior properties (Marcos et al., 2008). However, production and purification costs are very high and are currently limiting this possibility.

Plants, herbs, and spices, some of their derived essential oils, and substances isolated from different extracts, contain a large number of compounds that are known to inhibit the metabolic activity of many fungi (Valencia-Chamorro et al., 2011). Typically, the antifungal activity of these agents cannot be explained by a single specific mechanism but rather by the combined effect of the different chemical constituents that include high contents of terpenes, terpenoids, esthers, aldehydes, polyphenolic compounds, phenolic acids, and other aromatic constituents (Kuorwel et al., 2011). In general, essential oils have been one of the most studied compounds as antifungals because they have been known for centuries and some are classified as GRAS (Valencia-Chamorro et al., 2011). However, their commercial application is generally constrained by their strong flavor and odor and high reactivity with fruit constituents. This is one of the main reasons why their incorporation into edible coatings has arisen interest (Quirós-Sauceda et al., 2014).

In the last decades, a number of bacteria, yeast, and filamentous fungi have been isolated, identified and artificially used as biocontrol agents for the control of fruit postharvest pathogens (Dukare et al., 2019). The most important mechanisms of action of these antagonists are competition for nutrients and space, antibiotic production, production of cell wall lytic enzymes, production of antifungal volatile compounds, and induction of host resistance (Di Francesco et al., 2016; Dukare et al., 2019). Despite their antagonistic ability, the application of biocontrol agents often shows limitations such as the ability to yield stable products with high microbial viability, reduced adherence and survival once applied in the fruit, and high sensitivity to fluctuations in the storage conditions. In this sense, their incorporation into edible coatings can improve the survival of cell suspensions by providing nutrients, as well as the stability and dispersability in the fruit surface, providing a good adherence by acting as binding elements (Marín et al., 2017).

The use of metal-based nanoparticles represents a recent option for improving the properties of edible coatings, since they permit better mechanical resistance, transparency, controlled release, and more effective gas barrier properties (González-Reza et al., 2018; Zambrano-Zaragoza et al., 2018). The most widely-used inorganic components with antimicrobial activity that been studied for modifying the properties of edible coatings include montmorillonite, nano-SiOx, nano-TiO_2, nano-ZnO, iron oxides (Fe_3O_4, Fe_2O_3), as well as Ag nanoparticles, though it is important to note that the latter have some restrictions and are constantly under surveillance by the EFSA and other food safety authorities. Their mode of action has been mainly attributed to the generation of reactive oxygen species (ROS) that

[a] E-mail: palou_llu@gva.es

cause cell death (Azeredo, 2013). Although most of them have been evaluated as antibacterials, few studies have proven their activity as ingredients of edible coatings to control fungal postharvest diseases (Cerrillo et al., 2017).

Incorporation of antifungal ingredients into edible coatings

The success of antifungal edible coatings for fruits and vegetables is not only determined by the selection of the most appropriate coating-forming constituents and active ingredients for each pathogen and fruit host, but also by other factors such as type of coating application, environmental storage conditions, and bioavailability of the active ingredients. An effective incorporation of the antifungal agent to the coating formulation may be restricted by the chemical nature of the ingredients, which would affect the bioavailability in the coated fruit. In general, most of the antifungal ingredients, mainly organic acids and salts, antifungal proteins and peptides, and biocontrol agents are incorporated or imbibed directly into the coating matrixes or formulations. However, in many other cases, the physicochemical properties, the stability under certain conditions, or the low bioavailability of the antifungal ingredient requires its encapsulation into delivery systems, understood as those in which an active compound (core material) is entrapped into a carrier (wall material) (Acevedo-Fani et al., 2017). Furthermore, in other cases the incorporation is done through impregnation and/or modification of solid structures such as zeolites or clays (Quirós-Sauceda et al., 2014; Cerrillo et al., 2017). These techniques allow a significant improvement of solubility and bioavailability, facilitate controlled release, and protect sensitive bioactive compounds (González-Reza et al., 2018). Thus, for example, the incorporation of essential oils to coatings by encapsulation allows reducing some of the negative aspects that have their application to the fruit in aqueous solutions or as fumigants (volatiles), such as the loss of activity after drying due to their volatility and the generation of unpleasant aroma and flavor and/or fruit phytotoxicities at effective concentrations. In the case of other antifungal agents such as plant extracts and biocontrol agents, the encapsulation can improve the stability and dispersability, protecting them against environmental (e.g., ultraviolet radiation, desiccation, etc.) or chemical (e.g., ionic, chelation, etc.) interactions with the media that might affect their effectiveness (Quirós-Sauceda et al., 2014; Marín et al., 2017).

The most adequate encapsulation technique depends on the type and physical properties of the core and wall materials. Various polysaccharides (starch, cellulose, gum Arabic, carrageenan, alginate, xanthan, chitosan, etc.), proteins (whey protein, casein, gluten, gelatine, etc.) and lipids (phospholipids, fatty acids, waxes, etc.) may be used as wall materials. Table 2 shows the most widely used encapsulation techniques according to the types of wall materials.

Table 2. Wall materials and encapsulation process.

Type of wall material	Wall material	Encapsulation technique
Polysaccharides	Starch, cellulose, dextrin, maltodextrin, gum Arabic, carrageenan, alginate, xanthan, chitosan, agar, etc.	Spray and freeze-drying, coacervation, inclusion, ionotropic gelation (mainly gums, alginate, carrageenan)
Proteins	Whey protein, casein, gluten, gelatine, albumin, etc.	Emulsion-spray drying
Lipids	Phospholipids, fatty acids, waxes, fats, oils, diacylglicerols, polysorbates, etc.	Emulsion-liposome formation
Oligosaccharides	β–cyclodextrin	Inclusion

[a] E-mail: palou_llu@gva.es

Among them, the encapsulation processes most used for formulation of edible coatings are spray- and freeze-drying. Spray-drying requires emulsifying the substance into the encapsulating agent followed by drying the atomized emulsion in hot air. However, in some cases, the use of high temperature for the drying process can limit its use for heat sensitive ingredients such as essential oils, requiring other techniques such as freeze-drying (Quirós-Sauceda et al., 2014). Another possibility of encapsulation is the use of β-cyclodextrin as inclusion complex of antifungal agent. This is a cyclic oligosaccharide, consisting of (α-1,4)-linked α-d-glucopyranose units, with a hydrophilic outer surface and a lipophilic central cavity, able to form water-soluble inclusion complexes with many lipophilic poorly soluble compounds such as essential oils (Marques, 2010).

On the other hand, in order to improve the activity of the encapsulated active compounds incorporated into edible coatings, recent research works aim at their application at the nanoscopic scale (nanoemulsions, nanoparticles, etc.), which allows to increase the specific surface area of the active substance and, therefore, improve penetration, release efficiency, and/or absorption. In particular, nanoemulsions contain oil droplets with mean diameters between 20 and 200 nm. They are thermodynamically unstable but the rate of destabilization is lower than in conventional emulsions. Nanoemulsions also improve the biological activity of lipophilic compounds, such as essential oils, due to their capacity of increasing solubility and dispensability in water-based foods (Donsì and Ferrari, 2016). This would allow a better coverage by the antifungal essential oil of the pathogen infection sites in the fruit peel and, consequently, a reduction of the effective essential oil concentration needed, which in turn might translate in minimizing both the adverse impact of the essential oil on fruit sensory properties and the phytotoxicity risks (Acevedo-Fani et al., 2017). Similar principles apply to nanoencapsulation of antifungal agents in different core materials such as alginate, starch, arabic gum, chitosan or other biopolymers, used alone or in combination (Quirós-Sauceda et al., 2014). In the latter, the nanoencapsulation is achieved by multilayer deposition of oppositely charged biopolymers around the bioactive compound forming multilayer nanoemulsions and nanolaminates. These systems allow encapsulating both lipophilic and hydrophilic active compounds, locating them either in the lipid phase or in the interfacial coating (Acevedo-Fani et al., 2017).

In other cases, the incorporation of the antifungal agent is achieved by adsorption methods that modify the functionality of inorganic structures such as clays, metal oxides, and zeolites, to mention just a few (Azeredo, 2013; González-Reza et al., 2018). Although these nanostructures have been mainly studied in food packaging materials, their potential as antifungal carriers has also been explored for edible coatings. Among them, zeolites are the most widely used materials to incorporate Ag as antimicrobial agent for food applications. In Europe, the EFSA released a positive opinion concerning the use of two Ag-substituted zeolites in food contact surfaces, with silver migration into food matrices being restricted to 50 μg Ag per kg of food (Corrales et al., 2014). Therefore, this value should be currently considered as the limit to be incorporated into edible coatings for fruits and vegetables. In these antifungal edible coatings, the antifungal properties depend on the topology and composition of the zeolites, which affect Ag release (Cerrillo et al., 2017, 2018). In the case of clays or layered silicates, catione-exchange reactions have been used to replace the inorganic cations with organic surfactants, producing organically modified nanoclays with antimicrobial activity. These have been proven effective against Gram-positive and Gram-negative bacteria (Azeredo, 2013), but to our knowledge there is no information on their effect on postharvest pathogenic fungi.

[a] E-mail: palou_llu@gva.es

EVALUATION OF ANTIFUNGAL EDIBLE COATINGS

The performance of antifungal edible coatings should be evaluated considering the interactions among all the elements of the system: the target pathogen, the coating ingredients, particularly the antifungal compound, and the commodity and its postharvest handling. Therefore, coating development requires an initial optimization of coating formulations. In the case of synthetic composite coatings, this optimization is based on the chemical compatibility of the ingredients to achieve stable emulsions capable of forming homogeneous coatings. For a particular fruit commodity, the matrix and the non-antifungal ingredients of the coating are selected according to the coating's physiological activity in terms of reducing weight loss and increasing storability. For a particular pathosystem, the antifungal ingredients (food-grade chemical compounds or biocontrol agents) and concentrations are initially selected in accordance with previous information on their ability to control the target pathogen. Coating formulation is usually optimized on the basis of percent total solid content, total hydrocolloid-lipid content, and concentration of the antifungal component, but other parameters such as viscosity, pH, and wettability for the particular fruit commodity are also important (Valencia-Chamorro et al., 2008; Palou, 2018).

Formulations that are stable after incorporation of the selected antifungal ingredient at the selected concentration in the selected coating will then be tested to determine their ability to control the target postharvest disease. These evaluations can be performed in in vitro tests with films or, more commonly, directly in in vivo tests by coating fruit artificially or naturally inoculated with the pathogen. Incompatible emulsions solidify or show phase separation or undesirable physical characteristics. It can happen that a particular antifungal ingredient is not compatible at all with a particular coating, or that it is compatible only at concentrations or coating total solid contents below a specific threshold. For example, among 470 emulsions formulated with a hydroxypropyl methylcellulose (HPMC)-lipid (beeswax and shellac) matrix and about 30 antifungal food additives (mostly GRAS salts) and mixtures at a large range of concentrations, only 25 emulsions were selected for their high stability. They contained 6 to 8% solid content, 50% (dry basis) total lipid content, and a maximum of 2.5% (wet basis) food preservative (Valencia-Chamorro et al., 2008).

In vitro activity of films

The antifungal activity of stable antifungal coatings can be tested in vitro with coating films by means of the disk diameter test. Although films and coatings have the same chemical composition and sometimes are used as synonymous, they refer to different concepts according to their different purpose and utilization. Films are defined as a stand-alone thin layer of materials prepared by casting process and used as covers, wraps, or separation layers. They are usually used for determination of barrier, mechanical, and other properties of the coating formulation. On the other hand, fruit coatings involve the formation of films directly on the surface of the fruit to which they are intended to be applied (Palou et al., 2015).

For the disk diameter test, antifungal coating films are typically casted by pipetting the emulsion onto sterilized plastic plates and allowing them to dry for about 48 h at room temperature under aseptic conditions, inside a previously sterilized laminar flow hood. Dry films are peeled intact from the casting surface and aseptically cut into diameter disks using a sterile cork borer. Films from emulsion matrixes without the added antifungal ingredient are used as controls. Film disks are then aseptically transferred to the surface of agar culture medium Petri dishes (e.g., potato dextrose agar, PDA; dichloran rose-bengal chloramphenicol agar, DRBC) previously inoculated with spores of the target pathogen. The plates are refrigerated at 4-5°C for few hours to allow for the diffusion of film ingredients and then incubated at 20-25°C for 1-2 weeks. Usually, 3-4 agar plates (replicates) are prepared for each pathogen and film. The antifungal activity of the film is determined by

[a] E-mail: palou_llu@gva.es

measuring periodically during the incubation period the length of the inhibition zone around the film disk (Valencia-Chamorro et al., 2008). Results from disk diameter tests are illustrated in Figure 1 for the in vitro inhibition of the citrus pathogens *Penicillium digitatum* and *Penicillium italicum* by hydroxypropyl methylcellulose (HPMC)-lipid films containing different GRAS salts as antifungal ingredients.

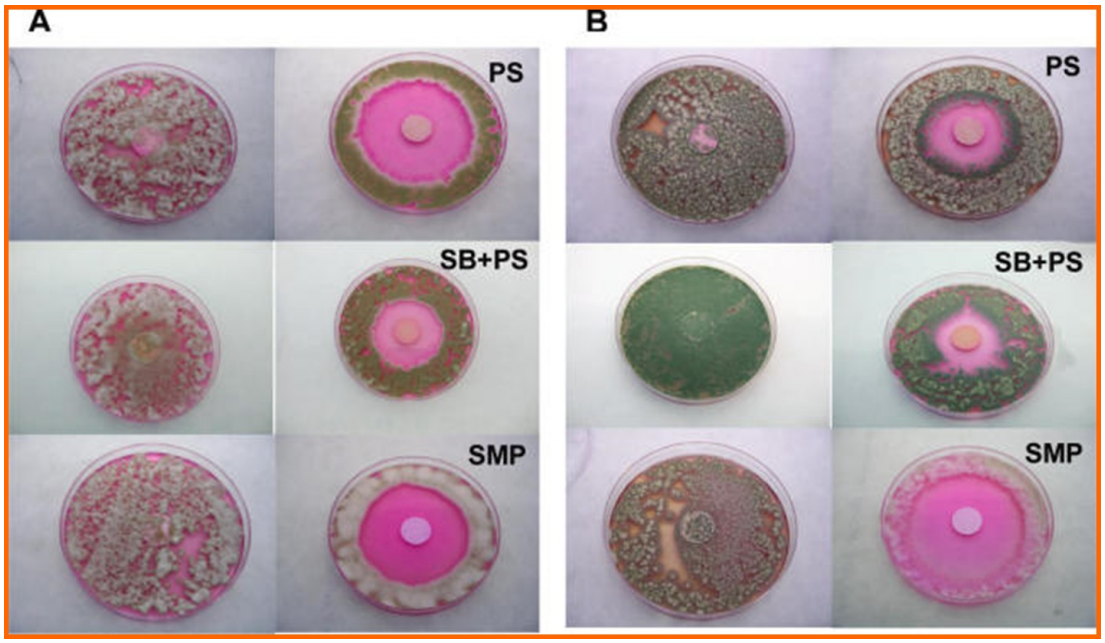

Figure 1. Disk diameter tests on DRBC agar plates for evaluation of the in vitro inhibition of (A) *Penicillium digitatum* and (B) *Penicillium italicum* by control HPMC-lipid films (left-side images) and HPMC-lipid films containing potassium sorbate (PS), a mixture of sodium benzoate and potassium sorbate (SB+PS) and sodium salt of methyl paraben (SMP) (right-side images).

In vivo disease control ability

Figure 2 represents a schematic diagram for the evaluation of disease control ability of antifungal coatings in in vivo tests, particularly for the control of Alternaria black spot on cherry tomato. Fresh fruit samples are selected, washed, artificially inoculated with the target pathogen, and, after about 24 h (curative activity), coated with the different coating treatments and allowed to dry on a mesh screen. Curative activity is usually assessed since the coatings will be commercially applied in the packinghouse to fruit already infected in the field (latent infections or wound infections occurring before or during harvest). Typically, fungal infections are resembled through artificial inoculation of the target pathogen in fruit peel wounds. For this, conidial suspensions of known concentration (10^4 to 10^6 spores mL^{-1}) are prepared from young PDA fungal cultures (incubation of 7–21 days at 20–25°C) dispersed in Tween 80®, filtered through two layers of cheesecloth to separate hyphal fragments, and adjusted to the desired concentration using a hemocytometer. Fungal inoculation is performed by applying a small volume (10–30 µL) of the conidial suspension to previously inflicted peel wounds. In other cases, the peel wound and the inoculation are performed at the same time by immersing the tip of a sterile stainless steel rod in the conidial suspension and inserting it into the fruit peel. Depending on the fruit size and shape, one or more peel wounds per fruit can be inflicted (Palou, 2018).

[a] E-mail: palou_llu@gva.es

Coatings are usually applied by fruit immersion for brief periods (10–30 s) (Valencia-Chamorro et al., 2009), but they can also be applied by pipetting a small amount of the emulsion (0.1–0.5 mL, depending on the commodity) onto each fruit and rubbing manually with gloved hands to mimic coating application in industrial packing line roller conveyors (Gunaydin et al., 2017). Control fruit are inoculated, but are uncoated or treated with coatings formulated without the antifungal ingredient. Depending on the experiment and the commodity, inoculated and treated fruit can be incubated at 20–25°C or cold-stored, similar to commercial postharvest handling, for a variable period of time (until most of control fruit are actually decayed). The usual sample size for these trials is 3–5 replicates of 20–25 fruit each. Treated fruit are commonly arranged in plastic cavity sockets on cardboard or plastic trays before incubation or cold storage. Disease incidence (percentage of infected wounds) and severity (lesion diameter, in mm or cm) and pathogen sporulation (percentage of lesions showing spores) are periodically determined during the incubation/storage period. Results can be also expressed as percent reductions with respect to control fruit, especially if results from several independent experiments are compiled (Palou, 2018).

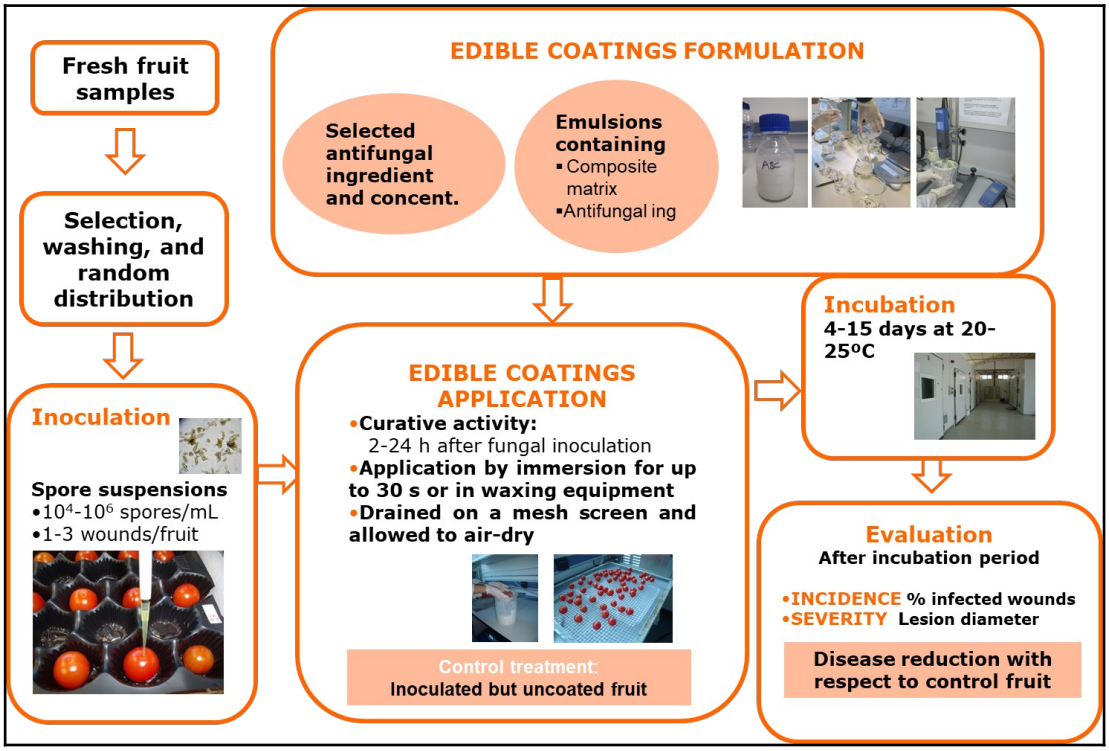

Figure 2. Methodological procedure for in vivo evaluation of the ability of antifungal edible coatings to control postharvest fungal diseases of fresh fruit. Illustrations refer to black spot of tomato caused by *Alternaria alternata*.

Effects on fruit quality and physiological behavior

Once the coatings with the greatest ability to control disease are identified, and as a last step for the selection of the most feasible antifungal coatings for each particular application, it is very important to determine the effect of coating application on the physiological behavior and overall quality of coated fruit. For this purpose, both physicochemical and sensory fruit quality attributes are periodically evaluated during and after cold storage and simulated periods of shelf life at 20°C (Valencia-Chamorro et al.,

[a] E-mail: palou_llu@gva.es

2009). Such evaluations are illustrated in Figure 3. Typically, the physicochemical fruit quality attributes that are assessed include weight loss (percent loss with respect to initial weight), peel color (with a colorimeter), fruit firmness (different types of measures with texturometers or penetrometers depending on the commodity), internal quality attributes, respiration and/or internal gas concentration (O_2 and CO_2 by gas chromatography), and overmaturation volatiles (ethanol and acetaldehyde contents by gas chromatography). Sensory fruit attributes such as flavor, off-flavors, and external and internal visual appearance should be evaluated by several trained judges with expertise in each particular commodity. In some cases, consumer tests by some non-trained individuals can also be of value.

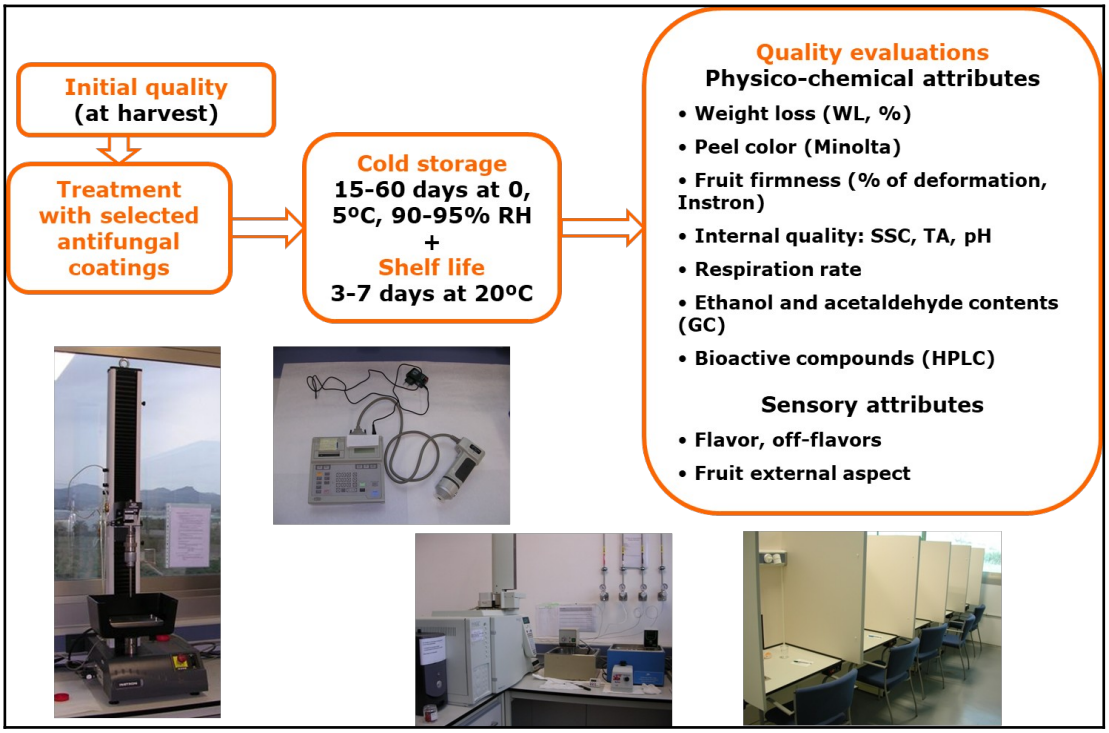

Figure 3. Illustrated methodological procedure for determination of the effect of coating application on overall quality and physiological behavior of cold-stored fresh fruit.

ACKNOWLEDGEMENTS

The "Agencia Estatal de Investigación" (AEI) from the "Ministerio de Ciencia, Innovación y Universidades" (MICINN) of Spain is acknowledged for funding research on this topic through the project RTA2015-00037-C02. IVIA is also acknowledged for funding research on this topic through the project IVIA-51910. The European Commission (FEDER programme) is also acknowledged for co-funding these projects.

Literature cited

Acevedo-Fani, A., Soliva-Fortuny, R., and Martin-Belloso, O. (2017). Nanostructured emulsions and nanolaminates for delivery of active ingredients: Improving food safety and functionality. Trends Food Sci. Technol. *60*, 12-22 http://doi.org/10.1016/j.tifs.2016.10.027

Ali, A., Zahid, N., Manickman, S., Siddiqui, Y., Alderson, P.G., and Maqbool, M. (2013). Effectiveness of submicron chitosan dispersions in controlling anthracnose and maintaining quality of dragon fruit. Postharvest Biol.

[a] E-mail: palou_llu@gva.es

Technol. *86*, 147–153 http://doi.org/10.1016/j.postharvbio.2013.06.027

Arnon, H., Zaitsev, Y., Porat, R., and Poverenov, E. (2014). Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. Postharvest Biol. Technol. *87*, 21–26 http://doi.org/10.1016/j.postharvbio.2013.08.007

Azeredo, H.M.C. (2013). Antimicrobial nanostructures in food packaging. Trends Food Sci. Technol. *30*, 56-69 http://doi.org/10.1016/j.tifs.2012.11.006

Bill, M., Sivakumar, D., Korsten, L., and Thompson, A.K. (2014). The efficacy of combined application of edible coatings and thyme oil in inducing resistance components in avocado (*Persea americana* Mill.) against anthracnose during post-harvest storage. Crop Prot. *64*, 159–167 http://doi.org/10.1016/j.cropro.2014.06.015

Cerrillo, J.L., Palomares, A.E., Rey, F., Valencia, S., Palou, L., and Pérez-Gago, M.B. (2017). Ag-zeolites as fungicidal material: Control of citrus green mold caused by *Penicillium digitatum*. Micropor. Mesopor. Mat. *254*, 69-76 http://doi.org/10.1016/j.micromeso.2017.03.036

Cerrillo, J.L., Palomares, A.E., Rey, F., Valencia, S., Pérez-Gago, M.B., Villamón, D., and Palou, L. (2018). Functional Ag-exchanged zeolites as biocide agents. ChemistrySelect *3*, 4676-4682 http://doi.org/10.1002/slct.201800432

Corrales, M., Fernández, A., and Han, J.H. (2014). Antimicrobial packaging systems. In: Innovations in Food Packaging, 2nd ed., J.H. Han, ed. (Waltham, MA, USA: Academic Press), p. 133-170 http://doi.org/10.1016/B978-0-12-394601-0.00007-2

de Oliveira, K.A.R., Berger, L.R., de Araújo, S.A., Cámara, M.P., and de Souza, E.L. (2017). Synergistic mixtures of chitosan and *Mentha piperita* L. essential oil to inhibit *Colletotrichum* species and anthracnose development in mango cultivar Tommy Atkins. Food Microbiol. *66*, 96–103 http://doi.org/10.1016/j.fm.2017.04.012

Dhumal, C.V. and Sarkar, P. (2018). Composite edible films and coatings from food-grade biopolymers. J. Food Sci. Technol.-Mysore. *55*, 4369-4383 http://doi.org/10.1007/s13197-018-3402-9

Di Francesco, A., Martini, C., and Mari, M. (2016). Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action? Eur. J. Plant Pathol. *145*, 711–717 https://doi.org/10.1007/s10658-016-0867-0

Donsì, F., and Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. J. Biotechnol. *233*, 106-120 https://doi.org/10.1016/j.jbiotec.2016.07.005

Dukare, A.S., Paul, S., Nambi, V.E., Gupta, R.K., Singh, R., Sharma, K., and Vishwakarma, R.K. (2019). Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. Crit. Rev. Food Sci. Nutr. *59*, 1498-1513 https://doi.org/10.1080/10408398.2017.1417235

EFSA. (2009). European Food Safety Authority, Panel on Dietetic Products, Nutrition and Allergies. Scientific opinion on the use of natamycin (E-235) as a food additive. EFSA Journal *7*, 1412.

EU CR. (2011). Commission Regulation (EU) No. 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Official Journal of the European Union *L295*, 1-177.

El-Ghaouth, A., Smilanick, J.L., Wisniewski, M., and Wilson, C.L. (2000). Improved control of apple and citrus fruit decay with a combination of *Candida saitoana* and 2-deoxy-D-glucose. Plant Dis. *84*, 249–253 https://doi.org/10.1094/PDIS.2000.84.3.249

El-Mohamedy, R.S., El-Gamal, N.G., and Bakeer, A.R.T. (2015). Application of chitosan and essential oils as alternatives fungicides to control green and blue moulds of citrus fruits. Int. J. Curr. Microbiol. Appl. Sci. *4*, 629-643.

González-Reza, R.M., García-Betanzos, C.I., Sánchez-Valdés, L.I., Quintanar-Guerrero, D., Cornejo-Villegas, M.A., and Zambrano-Zaragoza, M.L. (2018). The functionalization of nanostructures and their potential applications in edible coatings. Coatings *8*, 160 https://doi.org/10.3390/coatings8050160

Gunaydin, S., Karaca, H., Palou, L., de la Fuente, B., and Pérez-Gago, M.B. (2017). Effect of hydroxypropyl methylcellulose-beeswax composite edible coatings formulated with or without antifungal agents on physicochemical properties of plums during cold storage. J. Food Qual., *8573549* https://doi.org/10.1155/2017/8573549

Han, J.H. (2014). Edible films and coatings: a review. In: Innovations in Food Packaging, 2nd ed., J.H. Han, ed. (Waltham, MA, USA: Academic Press), p. 213-255 https://doi.org/10.1016/B978-0-12-394601-0.00009-6

Hassan, B., Chatha, S.A.S., Hussain, A.I., Zia, K.M., and Akhtar, N. (2018). Recent advances on polysaccharides, lipids and protein based edible films and coatings: A review. Int. J. Biol. Macromol. *109*, 1095-1107

[a] E-mail: palou_llu@gva.es

https://doi.org/10.1016/j.ijbiomac.2017.11.097

Jiwanit, P., Pitakpornpreecha, T., Pisuchpen, S., and Leelasuphakul, W. (2018). The use of *Aloe vera* gel coating supplemented with *Pichia guilliermondii* BCC5389 for enhancement of defense-related gene expression and secondary metabolism in mandarins to prevent postharvest losses from green mold rot. Biol. Control *117*, 43-51 https://doi.org/10.1016/j.biocontrol.2017.08.023

Kuorwel, K.K., Cran, M.J., Sonneveld, K., Miltz, J. and Bigger, S.W. (2011). Essential oils and their principal constituents as antimicrobial agents for synthetic packaging films. J. Food Sci. *76*, R164-R177 https://doi.org/10.1111/j.1750-3841.2011.02384.x

Marcos, J.F., Muñoz, A., Pérez-Payá, E., Misra, S., and López-García, B. (2008). Identification and rational design of novel antimicrobial peptides for plant protection. Annu. Rev. Phytopathol. *46*, 273–301 https://doi.org/10.1146/annurev.phyto.121307.094843

Marín, A., Atarés, L., and Chiralt, A. (2017). Improving function of biocontrol agents incorporated in antifungal fruit coatings: a review. Biocontrol Sci. Technol. *27*, 1220-1241 https://doi.org/10.1080/09583157.2017.1390068

Marques, H.M.C. (2010). A review on cyclodextrin encapsulation of essential oils and volatiles. Flavour Fragr. J. *25*, 313-326 https://doi.org/10.1002/ffj.2019

Martínez-Romero, D., Alburquerque, N., Valverde, J.M., Guillén, F., Castillo, S., Valero, D., and Serrano, M. (2006). Postharvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: A new edible coating. Postharvest Biol. Technol. *39*, 93–100 https://doi.org/10.1016/j.postharvbio.2005.09.006

Martínez-Romero, D., Zapata, P.J., Guillén, F., Paladines, D., Castillo, S., Valero, D., and Serrano, M. (2017). The addition of rosehip oil to *Aloe* gels improves their properties as postharvest coatings for maintaining quality in plum. Food Chem. *217*, 585–592 https://doi.org/10.1016/j.foodchem.2016.09.035

Moscoso-Ramírez, P.A., Montesinos-Herrero, C., and Palou, L. (2013). Characterization of postharvest treatments with sodium methylparaben to control citrus green and blue molds. Postharvest Biol. Technol. *77*, 128-137 http://doi.org/10.1016/j.postharvbio.2012.10.007

Mustafa, M.A., Ali, A., Manickam, S., and Siddiqui, Y. (2013). Ultrasound-assisted chitosan–surfactant nanostructure assemblies: towards maintaining postharvest quality of tomatoes. Food Bioprocess Technol. *7*, 2102-2111 http://doi.org/10.1016/10.1007/s11947-013-1173-x

Oliveira, P.D.L., de Oliveira, K.A.R., Vieira, W.A.D., Câmara, M.P.S. raiva, and de Souza, E.L. (2018). Control of anthracnose caused by *Colletotrichum* species in guava, mango and papaya using synergistic combinations of chitosan and *Cymbopogon citratus* (D.C. ex Nees) Stapf. essential oil. Int. J. Food Microbiol. *266*, 87–94 https://doi.org/10.1016/j.ijfoodmicro.2017.11.018

Ortega-Toro, R., Collazo-Bigliardi, S., Roselló, J., Santamarina, P., and Chiralt, P. (2017). Antifungal starch-based edible films containing *Aloe vera*. Food Hydrocoll. *72*, 1-10 https://doi.org/10.1016/j.foodhyd.2017.05.023

Palou, L. (2018). Postharvest treatments with GRAS salts to control fresh fruit decay. Horticulturae *4*, 46 https://doi.org/10.3390/horticulturae4040046

Palou, L., Ali, A., Fallik, E., and Romanazzi, G. (2016). GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest disease of fresh horticultural produce. Postharvest Biol. Technol. *122*, 41-52 https://doi.org/10.1016/j.postharvbio.2016.04.017

Palou, L., Valencia-Chamorro, S.A., and Pérez-Gago, M.B. (2015). Antifungal edible coatings for fresh citrus fruit: a review. Coatings *5,* 962-986 https://doi.org/10.3390/coatings5040962

Pérez-Gago, M.B., and Palou, L. (2016). Antimicrobial packaging for fresh and fresh-cut fruits and vegetables. In: Fresh-Cut Fruits and Vegetables: Technology, Physiology, and Safety, S. Pareek, ed. (Boca Raton, FL, USA: CRC Press), p. 403-452.

Quirós-Sauceda, A.E., Ayala-Zavala, J.F., Olivas, G.I., and González-Aguilar, G.A. (2014). Edible coatings as encapsulating matrices for bioactive compounds: a review. J. Food Sci. Technol.-Mysore. *51*, 1674-1685 https://doi.org/10.1007/s13197-013-1246-x

Romanazzi, G., Feliziani, E., Baños, S.B., and Sivakumar, D. (2017). Shelf life extension of fresh fruit and vegetables by chitosan treatment. Crit. Rev. Food Sci. Nutr. *57*, 579–601 https://doi.org/10.1016/j.lwt.2019.108574

Romanazzi, G., Feliziani, E., and Sivakumar, D. (2018). Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: Eliciting, antimicrobial and film-forming properties. Front. Microbiol. *9*, 2745 https://doi.org/10.3389/fmicb.2018.02745

[a] E-mail: palou_llu@gva.es

Sapper, M., Bonet, M., and Chiralt, A. (2019). Wettability of starch-gellan coatings on fruits, as affected by the incorporation of essential oil and/or surfactants. LWT - Food Sci. Technol. *116*, 108574. https://doi.org/10.1016/j.lwt.2019.108574

Sogvar, O.B., Saba, M.K., and Emamifar, A. (2016). *Aloe vera* and ascorbic acid coatings maintain postharvest quality and reduce microbial load of strawberry fruit. Postharvest Biol. Technol. *114*, 29–35 https://doi.org/10.1016/j.postharvbio.2015.11.019

Valencia-Chamorro, S.A., Palou, L., del Río, M.A., and Pérez-Gago, M.B. (2008). Inhibition of *Penicillium digitatum* and *Penicillium italicum* by hydroxypropyl methylcellulose-lipid edible composite films containing food additives with antifungal properties. J. Agric. Food Chem. *56*, 11270-11278 https://doi.org/10.1021/jf802384m

Valencia-Chamorro, S.A., Palou, L., del Río, M.A., and Pérez-Gago, M.B. (2011). Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: a review. Crit. Rev. Food Sci. Nutr. *51*, 872-900 http://doi.org/10.1080/10408398.2010.485705

Valencia-Chamorro, S.A., Pérez-Gago, M.B., del Río, M.A., and Palou, L. (2009). Curative and preventive activity of hydroxypropyl methylcellulose-lipid edible composite coatings containing antifungal food additives to control citrus postharvest green and blue molds. J. Agric. Food Chem. *57*, 2770-2777 https://doi.org/10.1021/jf803534a

Vieira, J.M., Flores-López, M.L., de Rodríguez, D.J., Sousa, M.C., Vicente, A.A., and Martins, J.T. (2016). Effect of chitosan-*Aloe vera* coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. Postharvest Biol. Technol. *116*, 88–97 https://doi.org/10.1016/j.postharvbio.2016.01.011

Wisniewski, M., Droby, S., Norelli, J., Liu, J., and Schena, L. (2016). Alternative management technologies for postharvest disease control: The journey from simplicity to complexity. Postharvest Biol. Technol. *122*, 3–10 https://doi.org/10.1016/j.postharvbio.2016.05.012

Zambrano-Zaragoza, M.L., González-Reza, R., Mendoza-Muñoz, N., Miranda-Linares, V., Bernal-Couoh, T.F., Mendoza-Elvira, S., and Quintanar-Guerrero, D. (2018). Nanosystems in edible coatings: A novel strategy for food preservation. Int. J. Mol. Sci. *19*, 705 http://dx.doi.org/10.3390/ijms19030705

Zapata, P.J., Navarro, D., Guillén, F., Castillo, S., Martínez-Romero, D., Valero, D., and Serrano, M. (2013). Characterisation of gels from different *Aloe* spp. as antifungal treatment: Potential crops for industrial applications. Ind. Crops Prod. *42*, 223–230 https://doi.org/10.1016/j.indcrop.2012.06.002

[a] E-mail: palou_llu@gva.es