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Review

Antifungal Edible Coatings for Fresh Citrus Fruit: A Review

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Abstract: According to their origin, major postharvest losses of citrus fruit are caused by weight loss, fungal diseases, physiological disorders, and quarantine pests. Cold storage and postharvest treatments with conventional chemical fungicides, synthetic waxes, or combinations of them are commonly used to minimize postharvest losses. However, the repeated application of these treatments has led to important problems such as health and environmental issues associated with fungicide residues or waxes containing ammoniacal compounds, or the proliferation of resistant pathogenic fungal strains. There is, therefore, an increasing need to find non-polluting alternatives to be used as part of integrated disease management (IDM) programs for preservation of fresh citrus fruit. Among them, the development of novel natural edible films and coatings with antimicrobial properties is a technological challenge for the industry and a very active research field worldwide. Chitosan and other edible coatings formulated by adding antifungal agents to composite emulsions based on polysaccharides or proteins and lipids are reviewed in this article. The most important antifungal ingredients are selected for their ability to control major citrus postharvest diseases like green and blue molds, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, and include low-toxicity or natural chemicals such as food additives, generally recognized as safe (GRAS) compounds, plant extracts, or essential oils, and biological control agents such as some antagonistic strains of yeasts or bacteria.

Keywords: *Citrus* spp.; postharvest; disease control; fruit quality; fungicide alternatives; edible coatings; chitosan; antifungal ingredients

1. Introduction

Citrus spp. (Rutaceae) are the most widely produced fruits in the world, with a global production that exceeded 123 million tons in 2013. The list of the most important producing countries is led by China, Brazil, the United States (USA), India, Mexico, and Spain. Fruit production is mainly devoted to juice extraction, but a considerable proportion is traded as fresh entire fruit for direct consumption. Spain is the leading country for exports of fresh produce [1]. From highest to lowest production, the most cultivated citrus species are oranges (*Citrus sinensis* L.), mandarins or tangerines (*Citrus reticulata* Blanco), including clementines (*Citrus clementina* hort. ex Tanaka), satsumas (*Citrus unshiu* Marcow.), and different hybrid mandarins, lemons (*Citrus limon* (L.) Burm. f.) and limes (*Citrus aurantiifolia* (Christm.) Swingle), and grapefruits (*Citrus paradisi* Macfad.). Postharvest handling of fresh fruit in citrus packing houses 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 packing house. These peel injuries are not only important for causing direct losses, but also for being infection sites for economically important postharvest fungal pathogens. Other pathological losses are caused by latent pathogens that infect flowers or young fruit in the grove but develop after harvest. Likewise, some postharvest physiological losses are originated in the field and others are caused by inappropriate handling or storage conditions in the packing house or the marketplace.

Postharvest treatments with conventional synthetic waxes and/or chemical fungicides such as imazalil (IMZ), thiabendazole (TBZ), sodium ortho-phenil phenate (SOPP) or other active ingredients have been used for many years and are still currently used in citrus packing houses to preserve fresh fruit, control postharvest decay, and extend fruit shelf life. Nevertheless, the continuous application of these treatments has arisen important problems for the citrus industry such as health and environmental issues associated with chemical residues or the proliferation of pathogenic resistant strains. Updated regulations from many countries are increasingly restricting the use of agrochemicals and every day more exports markets are demanding fruit with residue levels even lower than those established by official regulations. Due to this situation, research should focus on anticipating a scenario in which conventional chemical fungicides are not available. In such a context, satisfactory postharvest decay control may be accomplished by adopting integrated disease management (IDM) programs based on comprehensive knowledge of pathogen biology and epidemiology and consideration of all preharvest, harvest, and postharvest factors with influence on disease incidence. Among the antifungal postharvest treatments alternative to synthetic chemicals that are investigated worldwide for potential inclusion in IDM programs, in this article we review the development of antifungal edible coatings as a promising novel technology intended to confront two major concerns of citrus postharvest handling, the losses due to physiological problems and the losses due to pathological problems.

2. One Solution for Two Major Citrus Postharvest Problems

2.1. Physiological Problems

Citrus fruit are non-climacteric, hence their respiration rate and ethylene production do not exhibit remarkable increase along with changes related to maturity and ripening as in climacteric fruits. However, although they have a relatively long shelf life compared to other tropical and sub-tropical fruits, they may experience important physiological postharvest losses if they are not properly handled and stored. As with other horticultural products, major postharvest losses in citrus are caused by weight loss and physiological disorders.

Water loss of citrus fruit after harvest, although not considered a physiological disorder, is responsible for loss of quality and consumer acceptability, as it results not only in direct quantitative losses (loss of salable weight), but also in losses in appearance (wilting and shriveling) and softening. In addition, most physiological disorders that affect citrus fruit tend to be related to water loss [2]. Some rind disorders that may appear under optimal, non-chilling temperatures include peel pitting, stem-end rind breakdown (SERB), and shriveling and collapse of the stem-end button. Postharvest peel pitting at non-chilling temperatures is a severe disorder that affects fruit from several citrus cultivars worldwide. Although the causes of the disorder are not fully understood, evidence indicates that altered water relations in fruit peel is a major factor contributing to this disorder. In this sense, sudden changes from low to high relative humidity (RH) after harvest induced peel pitting in “Navelina” and “Navelate” oranges [3], “Marsh” grapefruit [4] or “Fallglo” tangerines [5]. Later studies confirmed the link between alterations in the osmotic and turgor potential in the flavedo (the outer pigmented layer of the peel) and albedo (the inner white layer of the peel) with the induction of postharvest peel pitting in citrus fruit [6]. SERB involves the collapse and subsequent darkening of the epidermal tissues around the stem-end rind of the fruit. The disorder is primarily associated with low RH, particularly during the postharvest period, although preharvest conditions seem to have a critical impact on the susceptibility of fruit to SERB. In some cases, SERB has been reported to be more severe when fruit are harvested from water-stressed trees compared to non-stressed trees or those presenting nutritional imbalances in terms of nitrogen and potassium [7–9]. Shriveling and collapse of the button tissue is an age-related breakdown due to cell weakening and dehydration of mature fruit. The symptoms can vary from discolored, dried out, and extensive collapse of the fruit rind to dehydration or wilting at the stem end where the rind is thinnest [10].

Another group of physiological disorders caused by storage of citrus fruit below sub-optimal temperatures but above the freezing point is chilling injury (CI). CI is characterized by the collapse of discrete areas of the peel that form sunken lesions that tend to coalesce [11]. CI in citrus fruit may appear in various forms depending on species and cultivar and exposure conditions (temperature and duration). For instance, typical symptoms in oranges, mandarins or grapefruits can be browning of the flavedo, appearance of dark sunken areas of collapsed tissue (pitting), or appearance of soft water-soaked areas (watery breakdown), while in lemons can be browning of the albedo or peteca (a special type of rind pitting) [2,10]. In general, although CI symptoms are due to fruit storage below their optimal temperature for relatively long periods, they usually develop upon transfer to higher temperatures.

The high sensitivity of citrus fruit to the induction of physiological disorders in the peel is in many cases triggered by mechanical damage during harvest and postharvest handling. Some of these disorders include “brush burn”, “zebra skin”, and “oleocellosis”, caused by rind abrasion, rough handling, or thorn punctures. Brush burn and zebra skin symptoms usually appear as superficial red/brown staining of the rind and, in the case of the latter, is associated with the position of the segments, whereas oleocellosis might result in dark sunken patches as cells collapse around oil glands [12].

2.2. Pathological Problems

The most important postharvest diseases of fresh citrus fruit are caused by filamentous fungi. According to the origin of the infections, they can be classified into two general groups, those that infect the fruit in the field and remain latent until their development after harvest, and those that infect the fruit through rind microwounds or injuries inflicted during fruit harvest, transportation, postharvest handling, and commercialization.

The most significant pathogens in the first group include *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. and *Phomopsis citri* H. Fawc. non Sacc. Traverso and Spessa, which cause the diseases commonly known as stem-end rots; *Alternaria citri* Ellis and N. Pierce in N. Pierce, the cause of alternaria rot or black rot; *Botrytis cinerea* Pers.:Fr., the cause of gray mold; *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz., the cause of anthracnose; and *Phytophthora citrophthora* (R.E. Sm. and E.H. Sm.) Leonian, which cause brown rot [13,14]. In general, the incidence of these pathogens is higher in citrus production areas with abundant summer rainfall, such as Florida or Brazil, because they require rain and humid weather for inoculum production and dispersal and subsequent fruit colonization and infection.

Among wound pathogens, the most economically important are *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer, which cause the diseases known as green and blue molds, respectively. These fungi are strict wound pathogens that can only infect the fruit through rind wounds inflicted in the field before or, more commonly, during fruit harvest, or after harvest during transportation, handling in the packing house, and commercialization. Green and blue molds are very important in all citrus production areas, including those with a Mediterranean-type climate, such as Spain, California, or South Africa, because one generation of *P. digitatum* or *P. italicum* is complete in rotten fruit in 7–10 days at usual ambient conditions of 20–25 °C and their spores are easily disseminated by air currents in large amounts [15]. Hence, the source of fungal inoculum in citrus orchards and packing houses is practically continuous during the season and the fruit can become readily contaminated [16]. Furthermore, healthy citrus fruit can become unmarketable when ‘soiled’ with conidia of these two fungi that are loosened during handling of diseased fruit [17]. No infection occurs if the fruit rind is intact because free conidia located on the peel surface are not able to germinate. However, the spores situated in injuries that rupture oil glands or penetrate into the albedo of the peel usually bring irreversible infection within 48 h at room temperatures [18]. The germination of both *Penicillium* species inside rind wounds requires free water, nutrients, and is stimulated by volatiles emitted from the host tissue [19,20]. *Geotrichum citri-aurantii* Ferraris E.E. Butler, the cause of sour rot; *Aspergillus niger* van Tiegh, the cause of Aspergillus rot; and *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill., the cause of Rhizopus rot, are other wound pathogens that can occasionally cause important postharvest losses under specific fruit handling and environmental conditions.

3. Generalities of Antifungal Edible Coatings

Fruit coating is a common practice in citrus packing houses to replace the natural waxes that can be removed during fruit washing and handling in the packing line. Citrus commercial coatings are generically known as waxes due to the fact that composition of initial formulations was based on paraffin wax or a combination of various other waxes such as beeswax or carnauba. Typically, they are anionic microemulsions containing resins and/or waxes, such as shellac, wood rosin, candelilla wax, carnauba wax, beeswax, polyethylene, or petroleum waxes. Their main purpose is to reduce fruit weight loss, shrinkage and improve appearance, but they can also reduce the incidence of CI or other citrus rind disorders [10,21]. In some cases, however, if the coatings excessively restrict gaseous exchange during fruit storage, fruit coating can adversely affect fruit flavor due to an overproduction of volatiles associated with anaerobic conditions [22,23]. In addition, commercial citrus waxes are often amended with synthetic chemical fungicides like IMZ, TBZ, or SOPP in order to control postharvest diseases, particularly green and blue molds.

Consumer interest in health, nutrition, and food safety combined with environmental concerns has increased the interest of many research groups to develop new natural, biodegradable, edible coating formulations to replace these currently used commercial waxes, thus avoiding the use in the formulations of synthetic components such as polyethylene wax, ammonia, or morpholine. The concept of antifungal edible coatings emerges when additional ingredients with antifungal properties (low-toxicity or food-grade preservatives, biocontrol agents, *etc.*) are incorporated into these biodegradable formulations in order also to replace the use of conventional chemical fungicides for postharvest disease control.

3.1. Films vs. Coatings

Although films and coatings have the same chemical composition and sometimes are used synonymously, they refer to different concepts according to their different purpose and utilization. Films are defined as a stand-alone thin layer of materials that can be used as covers, wraps, or separation layers. Films are usually prepared by casting process, and are mainly used for determination of barrier, mechanical, solubility, and other properties provided by certain film materials. On the other hand, food coatings involve the formation of films directly on the surface of the product to which they are intended to be applied. Thus, coatings form part of the final product [24].

3.2. Components and Types of Matrixes

In general, basic components or matrix components of edible coatings are hydrocolloids (proteins or polysaccharides), lipids (waxes, acylglycerols or fatty acids), and resins. Composite coatings or blends contain a combination of polysaccharides or proteins with lipids [25–27]. Plasticizers to enhance flexibility and extensibility, and emulsifiers or surfactants to improve the stability of emulsions may also be added as matrix components. These matrixes can be directly used in foods or act as carriers of other food additives such as antioxidants, nutraceuticals, flavorings agents, *etc.* that are included to modify the functionality of the coating [25,26,28–32]. In the particular case of antifungal edible coatings, the additional ingredients are food-grade antimicrobial compounds of different nature effective in preventing or reducing fungal growth. Characteristics of main components of matrixes are the following:

- Polysaccharides can form a continuous and cohesive matrix, which is related to their chemical structure by the association through hydrogen bonding of their polymeric chains [25]. Polysaccharides contain highly polar polymers with hydroxyl groups and present a good barrier to oxygen at low RH, but low moisture barrier due to hydrophilic properties [30]. Polysaccharide materials typically used to formulate edible or biodegradable coatings include cellulose, starch, pectin, chitosan, alginate, carrageenan, pullulan, and various gums [25,30,31,33–44].
- The ability of different proteins to form edible coatings is highly dependent on their molecular characteristics: molecular weight, conformation, electrical properties, flexibility, and thermal stability [45]. Edible coatings based on proteins usually exhibit good gas barrier characteristics, but poor water barrier characteristics due to their hydrophilic character [28,46,47]. Common proteins used for edible coating formulation include, among others, corn zein, casein, wheat gluten, soy protein, whey protein, keratin, or rice bran protein [26,30,47–52]. It is important to note that some people may present allergies or protein intolerances, e.g., to wheat gluten (celiac disease) or milk protein, which could restrict the use of protein-based coatings.
- Edible films and coatings based on hydrophobic substances, such as lipids and resins, are indicated to provide a barrier to moisture and gloss to food surfaces. However, since these materials are not polymers they form films and coating with poor mechanical properties and opaque characteristics [53]. Hydrophobic substances used as components of edible coatings include a variety of animal and vegetal native oils and fats, e.g., peanut, coconut, palm, lard, tallow, *etc.*; fractionated, concentrated, and/or reconstituted oils and fats, e.g., fatty acids, mono-, di-, and triglycerides, cocoa butter substitutes, *etc.*; hydrogenated and/or transesterified oils, e.g., margarine, shortenings, *etc.*; natural vegetal and animal waxes, e.g., beeswax, candelilla, carnauba, jojoba bees, rice bran, *etc.*; non-natural waxes, e.g., paraffins, oxidized or non-oxidized polyethylene; and natural resins, e.g., asafoetida, benjoin, chicle, guarana, myrrhe, olibanum (incense), opoponax, shellac resins, wood rosin, *etc.* [41,54–57].
- Composite coatings or blends typically contain hydrocolloid components, *i.e.*, protein and/or polysaccharides, and lipids in order to combine the advantages of both types of components. Composite coatings can be produced as either bi-layer or stable emulsions. In bi-layer coatings, the lipid forms a second layer over the protein or polysaccharide layer. In emulsion coatings, the lipid is dispersed and entrapped in the supporting matrix of protein or polysaccharide [57–59]. In this type of coatings, the efficiency of lipid materials depends on the lipid structure, its chemical arrangement, hydrophobicity, physical state, and its interaction with other components of the film [53].
- Plasticizers are low molecular weight compounds of small size, high polarity, high amount of polar groups per molecule, and great distance between polar groups within a molecule. They are added to edible coating materials to decrease the intermolecular forces between polymer chains, which results in greater flexibility, elongation, toughness, and permeability [28,30,32,60]. They are, therefore, particularly indicated to form stable emulsions and improve mechanical properties when hydrocolloids and lipids are combined. Common plasticizers used for edible coatings include sucrose, glycerol, sorbitol, propylene glycol, polyethylene glycol, fatty acids, and monoglycerides. Water can also act as a plasticizer for polysaccharide and protein coatings [61].

- Emulsifiers or surfactants are agents of amphiphilic nature that interact at the water-lipid interface and reduce surface tension between the dispersed and continuous phases to improve the stability of the emulsion when hydrocolloids and lipids are combined [30]. Moreover, emulsifiers added to coating formulations promote good surface wetting, spreading, and adhesion of the coating to the food surface. Typical emulsifiers used on edible coatings are fatty acids, ethylene glycol monostearate, glycerol monostearate, esters of fatty acids, lecithin, sucrose ester, and sorbitan monostearate, or polysorbates (tweens).

3.3. Functional Properties of Edible Coatings

The most important functional properties of edible films and coatings are edibility and biodegradability; migration, permeation, and barrier functions; and physical and mechanical protection. Such properties allow their use for food quality preservation and shelf-life extension. Furthermore, as carriers of active compounds that can be released in a controlled way, they can also provide antimicrobial activity and be applied for decay control and safety enhancement [26,29,33,35,47,62]. Edibility and biodegradability are the most beneficial characteristics of edible films and coatings. Edibility should be achieved by using food-grade ingredients for all coating components. Moreover, the whole process, facilities, and equipment should be feasible for food processing, and all components should be biodegradable and environmentally safe [24,26,63].

Edible coatings maintain food integrity and can protect coated food products against bruising, tissue damage and, in general, physical injury caused by impact, pressure, vibrations, and other mechanical factors. The ability of edible films and coatings to protect food against mechanical damage is usually assessed by determining film tensile properties such as Young's modulus (YM), which determines film stiffness as determined by ratio of pulling force/area to degree-of-film-stretch, tensile strength (TS), which indicates the pulling force per film cross-sectional area required to break the film, and elongation at break (E), which gives the degree to which the film can stretch before breaking and it is expressed as a percentage [1,10]. Other standardized mechanical examinations include compression strength, puncture strength, stiffness, tearing strength, burst strength, abrasion resistance, adhesion force, and folding endurance [24,29,35,47].

Since one of the main functions of edible coatings is to act as a protective barrier to environmental moisture, gases, flavors, aromas, or oils [26,64], other properties that are often determined on stand-alone films are water vapor permeability (WVP), oxygen permeability (OP), carbon dioxide permeability, and flavor permeability. Aroma and oil permeability are also very important for many foods but have generally received less attention. In other cases, other properties of interest are water solubility, gloss, and color.

The general protective functions of edible films and coatings may be widened with the addition to the matrixes of additional ingredients that provide new functionalities. These food additives include antioxidants, colorants, flavors, nutraceuticals, nutrients, and antimicrobial and particularly antifungal compounds [25,28–30,62,65].

3.4. Types of Antifungal Ingredients

With the exception of chitosan that presents inherent antimicrobial activity against a wide range of foodborne fungi, the antifungal effect of biopolymer-based coatings is usually achieved by the incorporation of active antimicrobial compounds to the coating formulation. Another exceptional case are gels and aqueous extracts from the leaves of the plant *Aloe vera*, which are edible and can also be used to coat fresh or minimally processed horticultural products. These coatings have been basically used for physiologic preservation of fruit after harvest, but they are also known for their antimicrobial activity [66–68]. However, in contrast to chitosan-based coatings, there is very limited information on the performance of coatings based on *A. vera* gels against fungi causing postharvest decay of citrus fruit. Early work by Saks and Barkai-Golan [69] showed significant activity of *A. vera* gels against *P. digitatum in vitro* and against green mold on grapefruits artificially inoculated with this fungus. Similar results were obtained in more recent research by [70], who found that an *A. vera*-based coating reduced green and blue molds on "Kinnow" mandarins and positively affected fruit quality during cold storage.

According to their nature, the antifungal compounds that can be added as additional ingredients to edible coatings can be classified into three categories: (1) synthetic food preservatives or GRAS (generally recognized as safe) compounds with antimicrobial activity, which include some inorganic and organic acids and their salts (carbonates, propionates, sorbates, benzoates, *etc.*), parabens (methyl and ethyl parabens) and their salts, *etc.*; (2) natural compounds such as essential oils or other natural plant extracts (cinnamon, capsicum, lemongrass, oregano, rosemary, garlic, vanilla, carvacrol, citral, cinnamaldehyde, vanillin, grape seed extracts, *etc.*) [33]; and (3) antimicrobial antagonists as biological control agents (yeasts, bacteria, and even some filamentous fungi). As they need to be allowed as food ingredients, all compounds in the first two categories should be synthetic or natural substances with known and minimal toxicological effects on mammals and the environment and they must be classified as food-grade additives or GRAS compounds by relevant regulations [71,72]. On the other hand, biocontrol agents must comply with strict and complex specific legislation, which considerably differs among different countries [73].

4. Chitosan and Chitosan-Based Citrus Coatings

Chitosan is a natural biopolymer with antimicrobial activity that has the property to form edible films and coatings [74]. 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 [35,75]. Chitosan is produced commercially with different deacetylated grades and molecular weights, which are related to their functional properties and antimicrobial effects. Chitosan has exhibited high antimicrobial activity against a range of spoilage microorganisms, including fungi, yeasts, and bacteria. Particularly, its beneficial effect for postharvest disease reduction has been reported for a wide variety of fresh horticultural produce including citrus, apples, mango, grapes, strawberries, blueberries, lettuce, carrots, or tomatoes. 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 the presence of other ingredients in the chitosan coating [25,35,76–79]. In addition to its antimicrobial and antifungal activity, chitosan has been considered as a good candidate for postharvest treatment and long-term storage of fresh fruits and vegetables because of other important properties, such as lack of toxicity to mammals and consequent edibility, biodegradability, biocompatibility with many other compounds, and multifunctionality, greatly derived from its capacity to form coatings [62,75].

Chitosan and derivatives are currently the most assayed antifungal edible coatings for postharvest preservation of fresh citrus fruit. They have been investigated either alone or formulated with other additional antifungal ingredients. Table 1 summarizes the most important research applications, to date, of chitosan and chitosan-based coatings, including the additional antimicrobial agents, if any, the target pathogen, an assessment of the antifungal activity, and the literature reference.

Table 1. Antifungal chitosan-based edible composite coatings applied to fresh citrus fruit.

Citrus Fruit	Coating	Concentration	Antimicrobial Agent	Target Pathogen	Antimocrobial Activity [a]	Reference
“Navel” orange, Lime	Chitosan	2, 4, 6, 8 g·L^{-1}	Lemongrass oil, Citral (6, 8 mL·L^{-1})	*Penicillium digitatum*, *Penicillium italicum*	+	[80]
	Chitosan	–	Lemongrass oil, Citral	*P. digitatum*, *P. italicum*	+	
“Satsuma” mandarin	Chitosan	1.0%	–	*P. digitatum*	+	[81]
	Chitosan	1.0%	Clove oil (0.5 mL·L^{-1})	*P. digitatum*	-	
“Or” and “Mor” mandarins, “Star Ruby” grapefruit	CMC, chitosan	1.0%, 1.5%	–	–	ND	[82]
“Jincheng 447” orange	Oligochitosan	1.5%	–	*Colletotrichum gloeosporioides*	+	[83]
“Valencia” orange	Chitosan	0.5%	–	*P. digitatum*	+	[84]
“Washington Navel” orange	Chitosan	0.5%	–	*P. digitatum*	+	–
“Femminello” lemon	Chitosan	0.5%	–	*P. digitatum*	+	–
“Marsh Seedlees” grapefruit	Chitosan	0.5%	–	*P. digitatum*	+	–
“Navel Powell” orange	Chitosan	2%	Bergamot, thyme and tea tree oils	*P. italicum*	+	[85]
“Valencia” and ‘Pêra Rio” oranges	Chitosan, Chitosan + TBZ	2%	–	*Guignardia citricarpa*	+	[34,86]

Table 1. *Cont.*

Citrus Fruit	Coating	Concentration	Antimicrobial Agent	Target Pathogen	Antimocrobial Activity [a]	Reference
Lime	Chitosan	2, 4, 6, 8 g·L^{-1}	Citral (0, 2, 3, 4, 5 mL·L^{-1})	*Geotrichum citri-aurantii*	+	[87]
"Navel" orange	Chitosan	2%	–	*P. digitatum*, *P. italicum*	+	[77]
"Murcott" tangor	Chitosan (high MW)	0.05%, 0.1%, 0.2%	–	*P. digitatum*, *P. italicum*, *Botrydiplodia lecanidion*, *Botrytis cinerea*	+	[88]
	Chitosan (low MW)	0.05%, 0.1%, 0.2%	–	*P. italicum*, *B. lecanidion*, *B. cinerea*	+	
"Tankan" tangor	Chitosan	0.05%–0.2%	–	*P. digitatum*, *P. italicum*	-	[78]
Lemon	Chitosan	1 mg·mL^{-1}	–	*P. digitatum*	-	[89]
"Fortune" mandarin, "Valencia" orange	Biorend® (chitosan comercial product)	–	–	–	+	[90]
"Eureka" lemon	Glycolchitosan	0.2%	*Candida saitoana* (10^{8} CFU·mL^{-1})	*P. digitatum*	+	[91,92]
"Washington Navel" orange	Glycolchitosan	0.2%	*C. saitoana* (10^{8} CFU·mL^{-1})	*P. digitatum*	+	–

CMC = carboxymethil cellulose, MW = molecular weight, TBZ = thiabendazole; [a] Antimicrobial activity: "+" = inhibition, "-" = no inhibition, ND = not determined.

4.1. Stand-Alone Chitosan Coatings

The application of chitosan to lemon fruit prior to inoculation with *P. digitatum* resulted in a near absence of fungal development in inoculated rind wounds [89]. This worker suggested that the treatment had the ability to induce the transcriptional activation of defense genes leading to the accumulation of structural and biochemical compounds at strategic sites. Chitosan against green mold caused by *P. digitatum* was tested at concentrations from 0.02% to 0.5% in both *in vitro* and *in vivo* assays. It was found in the *in vitro* tests that chitosan at concentrations higher than 0.1% totally inhibited the pathogen growth, while in the *in vivo* assays chitosan applied at the concentration of 0.5% significantly reduced green mold on "Valencia" and "Washington Navel" oranges, "Femminello" lemons and "Marsh Seedless" grapefruits. The antifungal effects of chitosan were related to direct fungitoxic activity against the pathogen [84]. In another study [77], chitosan at 2% was applied to "Navel" oranges 24 h before inoculation with *P. digitatum* or *P. italicum* and it was observed that, after 23 days at 20 °C, disease incidence (proportion of infected fruit) and severity (lesion diameter) were significantly lower on coated samples than on uncoated controls. Chitosan antifungal activity was higher against blue mold than against green mold. It was pointed out, moreover, that chitosan application enhanced the activities of several enzymes such as peroxidase (POD) and superoxide dismutase (SOD), and increased the levels of glutathione (GSH) and hydrogen peroxide (H_2O_2). The effects of low (15 kDa) and high (357 kDa) molecular weight chitosan coatings, applied at 0.05% to 0.2%, on the development of green and blue molds and the quality of "Murcott" tangerines were studied by Chien, *et al.* [88]. Low molecular weight chitosan at 0.2% showed effective antifungal activity against both molds and improved firmness, titratable acidity, ascorbic acid level, and water content of coated fruit. Moreover, the performance of low molecular weight chitosan was comparable with that of the synthetic chemical fungicide thiabendazole. In another research, the antifungal activity of chitosan (0.05%–0.2%) with two different molecular weights (92.1 kDa and 357.3 kDa) against *P. digitatum* and *P. italicum* and the effect on postharvest quality of "Tankan" fruit, were investigated [78]. The performance of chitosan depended on its type and concentration. Chitosan effectively reduced the growth of the pathogens and the percentage of decayed fruit during incubation at 24 °C. On the other hand, fruit treated with chitosan exhibited good chemical and physical properties and less decay than control fruit during long-term refrigerated storage.

Deng, *et al.* [83] studied the effect of oligochitosan (1.5%), a derivate product from the enzymatic hydrolysis of chitosan, on the development of anthracnose on oranges inoculated with *C. gloeosporioides*. The results showed lower disease incidence and severity on oligochitosan-treated than on control fruit. The authors concluded that the treatment had the ability to induce strong disease resistance in citrus fruit during storage by increasing the contents in the fruit peel of constitutive natural antifungal compounds and the activity of defense enzymes. Glycolchitosan, another chitosan derivative, was also tested, alone or in combination with other treatments, for the control of green mold on oranges and lemons [91].

Two citrus varieties, "Fortune" mandarins and "Valencia" oranges were coated with a commercial chitosan-based coating product (Biorend®, IDEBIO S.L., Salamanca, Spain), and its effects on decay, fungistatic action, and fruit quality attributes were studied. The ripening process and decay development in coated fruit were monitored by means of the magnetic resonance imaging (MRI) technique [90]. The authors concluded that this chitosan product delayed maturity and reduced decay on both citrus species, allowing the effective preservation of "Fortune" mandarins and "Valencia" oranges for 6 and 22 weeks, respectively.

Work in Brazil [86] showed the potential of chitosan coatings to prevent the development of black spot, an important quarantine disease caused by the pathogen *Phyllosticta citricarpa* (McAlpine) van de Aa, on "Valencia" oranges stored at either 25 °C for eight days or 3 °C for 21 days. The authors discussed that chitosan application stimulated disease resistance mechanisms in the fruit rind.

4.2. Chitosan Coatings Amended with other Antifungal Ingredients

The possibility of enhancing the antifungal activity of chitosan coatings by combining them with other antifungal treatments has been explored as well. Such complementary antifungal treatments can be of different nature (physical treatments [93], chemical fungicides [34], food additives [91], biocontrol antagonists [94,95], *etc.*) and may be applied to citrus fruit before or after the application of chitosan-based coatings. However, in this review we focus on the addition of other antifungal agents to chitosan coating matrixes that are afterwards applied to the fruit as a unique postharvest treatment.

In general, chitosan-amended coatings allow a gradual release of preservatives and provide additional properties for fungal growth inhibition and fruit quality maintenance [90]. The most important of these additional antifungal ingredients are essential oils. In few cases, biocontrol agents have been tested as well.

The antifungal activity, either *in vitro* or *in vivo*, of many essential oils extracted from plants or fruits such as birch, bergamot, cumin, cinnamon, citrus, clove, lemongrass, oregano, thyme, or tea tree against major citrus pathogens, particularly *P. digitatum* and *P. italicum*, has been reported [15,96]. Among them, only a limited amount has been tested as potential ingredients of chitosan coatings. Chitosan combined with citral or lemongrass oil (at 3 $mL \cdot L^{-1}$ or 4 $mL \cdot L^{-1}$) significantly reduced the mycelial growth and spore germination of *P. digitatum* and *P. italicum* in *in vitro* tests, while when applied to oranges and limes also provided a considerable green and blue mold reduction on fruit stored at either 20 °C or refrigeration temperatures [80]. Faten [87] reported good results with a combination of citral and chitosan for the control on lime fruit of sour rot, caused by the pathogen *G. citri-aurantii*. Reductions of disease incidence and severity as large as 89.5% and 93.5%, respectively, were obtained after the application of this coating containing chitosan (at 6 $g \cdot L^{-1}$ or 8.0 $g \cdot L^{-1}$) and citral (at 4 $mL \cdot L^{-1}$ or 5 $mL \cdot L^{-1}$). Both preventive (fruit coating followed by fungal inoculation) and curative (inoculation followed by coating) activity of chitosan coatings, containing or not essential oils from bergamot, thyme, or tea tree, were determined on "Powell" navel oranges against *P. italicum* [85]. It was found that, in general, the amended coatings were more effective than chitosan alone in reducing blue mold and that preventive activity was higher than curative activity. Among the tested oils, thymol showed the greatest antifungal activity when incorporated with the chitosan coating. Moreover, fruit quality attributes (acidity, pH, soluble solids, juice percentage, weight loss, firmness, color parameters, and respiration rate) of coated samples did not present any relevant changes during long-term cold storage. The inhibition by vapor contact of *A. niger* and *P. digitatum* by selected concentrations of Mexican oregano, cinnamon, or lemongrass essential oils added to chitosan, amaranth, or starch edible films was evaluated in another research [97]. The results showed that chitosan edible films incorporating Mexican oregano or cinnamon oils inhibited the two fungi at lower concentrations than those required for amaranth and starch edible films, and it was concluded that chitosan improved the release of the oil antimicrobial compounds. Sánchez-González, *et al.* [98] studied the addition of different concentrations of tea tree essential oil into chitosan films. Mechanical and optical properties and WVP of dry films, and antimicrobial activity against *P. italicum* were determined. They reported that films with a ratio of tea tree essential oil:chitosan of 1:2 provided a complete inhibition of fungal growth after five days of storage at 10 °C. Nevertheless, no every study reported synergistic activity from the addition of essential oils to chitosan coatings. Very recent work by Shao, *et al.* [81] showed that chitosan combined with clove oil was not significantly more effective than chitosan alone for the control of green mold on "Satsuma" mandarins artificially inoculated with *P. digitatum*. Furthermore, the high antifungal activity observed in *in vitro* tests was not obtained in *in vivo* tests, despite that the combination of chitosan and clove oil enhanced the activity of some fruit defense enzymes.

With reference to biocontrol agents as additional antifungal ingredients in chitosan-based coatings, El-Ghaouth, *et al.* [92] assessed the effectiveness of a 0.2% glycolchitosan coating formulated with the antagonistic yeast *Candida saitoana* against citrus green mold. They observed that this bioactive coating was superior to stand-alone glycolchitosan and *C. saitoana* treatments in controlling green mold on different orange and lemon cultivars, and the control level was equivalent to that with IMZ. The biocoating also reduced the incidence of stem-end rot caused by the pathogens *L. theobromae* or *P.citri* on naturally infected “Valencia” oranges, although in this case disease control was lower than that with IMZ. The maximum efficacy against green mold on “Eureka” lemons artificially inoculated with *P. digitatum* was obtained when citrus fruit were pretreated with the food additive sodium carbonate followed by the application of the glycolchitosan coating containing *C. saitoana* [91].

Along with the addition of essential oils or biocontrol agents, another system that has been evaluated to improve the activity of chitosan is the development of bilayer coatings comprised of chitosan and another natural polymer. This is the case of an edible composite bilayer coating formulated with carboxymethyl cellulose (CMC) and chitosan [82]. These workers applied this new coating to mandarins, oranges, and grapefruits and observed beneficial effects for fruit postharvest quality preservation. However, its effect on postharvest decay reduction was not studied.

5. Citrus Coatings Formulated with GRAS salts

Among the different GRAS compounds, organic acids and their salts are the most common synthetic antimicrobial agents tested in food systems [96]. In research conducted in our laboratory, Valencia-Chamorro, *et al.* [99] developed and optimized hydroxypropyl methylcellulose (HPMC)-lipid edible composite films formulated with a wide variety of food additives or GRAS compounds (mineral salts, organic acid salts, parabens, *etc.*) to inhibit the *in vitro* growth of the pathogens *P. digitatum* and *P. italicum*. Afterwards, the curative activity of selected coatings against green and blue molds was tested *in vivo* on oranges and mandarins artificially inoculated with the pathogens, coated, and incubated at 20 °C for a shelf life period of seven days [100]. It was found that coatings containing the GRAS salts potassium sorbate (PS), sodium benzoate (SB), sodium propionate (SP) and their mixtures were the most effective for disease reduction. Subsequent studies confirmed the antifungal activity and good effects on preservation of fruit quality of HPMC-lipid edible coatings formulated with PS, SB, SP, or their mixtures on long-term cold-stored “Valencia” oranges [101], “Ortanique” mandarins [102] and “Clemenules” mandarins [103]. In general, these coatings reduced fruit weight loss and maintained firmness without adversely affecting the overall sensory quality of coated fruit.

In research to explore the ability of GRAS salts to substitute the use of synthetic fungicides in conventional citrus commercial waxes, Youssef, *et al.* [104] reported that the salts sodium carbonate (SC) and bicarbonate (SBC), potassium carbonate (PC) and bicarbonate (PBC), ammonium bicarbonate (ABC), and PS incorporated into a commercial wax at a concentration of 6% (*w*/*v*) significantly reduced the incidence of postharvest diseases on naturally infected “Tarocco” and “Valencia” oranges and “Comune” clementines during cold storage followed by one week of shelf life at 20 °C. Among them, wax containing PS was the most effective to reduce decay in all tested cultivars.

6. Citrus Coatings Formulated with Essential Oils

Among plant-derived natural compounds, many essential oils have proven antimicrobial activity against different important fungal pathogens, thus gaining popularity as potential ingredients of fruit coatings. The essential oils most studied for this purpose include clove, oregano, thyme, nutmeg, basil, mustard, and cinnamon oils, and some of them have been classified as GRAS by regulators [105].

Essential oils, as stand-alone treatments for fruit preservation, can be applied as volatiles in modified atmosphere packages or prepared as aqueous solutions from plant extracts obtained with different solvents. In any case, research has frequently shown that the efficacy of these compounds in *in vivo* experiments with fruit is lower than that observed in *in vitro* tests and, in some cases, they can be phytotoxic or induce negative sensory properties to treated commodities [96,106]. Therefore, despite their important antifungal activity, commercial implementation of essential oils as stand-alone postharvest treatments is strongly restricted and their use as ingredients of edible coatings might be a potential solution for these application problems.

Good antifungal activity was achieved with an edible coating formulated with a CMC matrix amended with a stem extract of the herb *Impatiens balsamina*. The application of this coating significantly reduced total decay incidence and preserved fruit weight without adverse effects on internal quality on "Newhall" oranges stored at 5 °C for up to 100 days [107]. The mode of action of the coating was attributed to an increase of the activities of important fruit defense enzymes such as POD, SOD, chitinase (CHI), and β-1,3-glucanase (GLU). Velásquez, *et al.* [108] compared in laboratory trials the effect of a pectin-based edible coating amended with essential oil at different concentrations with that of commercial waxes for disease control on "Valencia" oranges inoculated with *Penicillium* sp. The pectin coating containing essential oil at 1.5% reduced decay by 83% and satisfactorily extended the shelf life of coated fruit stored at 23 °C. Nevertheless, both amended coatings and commercial waxes were not effective on fruit stored at low temperatures.

In other research works, commercial citrus waxes have been formulated with essential oils as an alternative to the use of synthetic chemical fungicides as components of these waxes. In semi-commercial and commercial trials, essential oils from the plants *Mentha spicata* and *Lippia scaberrima* incorporated into different commercial citrus waxes resulted in significant preventive activity against green mold on "Valencia" and "Tomango" oranges. Specifically, after six days of incubation at ambient temperature, essential oil from *L. scaberrima* added at 2500 $mL \cdot L^{-1}$ into a carnauba wax reduced by about 60% the incidence of green mold compared to control fruit coated with the carnauba wax without essential oil [109]. Moreover, the amended waxes significantly decreased orange weight loss and maintained overall fruit quality. The effectiveness of the amended coatings was related to the strong inhibitory activity against *P. digitatum* of one of the most important components of the essential oil, the terpenoid R-carvone. In laboratory tests conducted in Spain, the application of wax coatings formulated with the essential oils carvacrol or thymol satisfactorily reduced green mold incidence on lemons artificially inoculated with *P. digitatum* and, at the same time, reduced the lemon respiration rate and ethylene production [110]. Further semi-commercial trials conducted in lemon packing lines confirmed that these essential oil-amended waxes were as effective as waxes containing the conventional fungicide IMZ in reducing lemon postharvest decay and total aerobic microflora present on fruit surface. In addition, fruit weight loss and rind softening and color were equally preserved by both types of coatings [111].

In another work, citral was incorporated into a commercial carnauba wax and a concentration 10 times the minimum fungicidal concentration obtained in *in vitro* studies was required to significantly decrease the incidence of green mold on citrus fruit coated and incubated at 25 °C for 6 days [112]. Octanal, another component of citrus essentials oils, has also been recently evaluated as active ingredient of commercial waxes for postharvest coating of citrus fruit. Tao, *et al.* [113] determined in *in vitro* tests that the minimum fungicidal concentration (MFC) of this compound against *P. digitatum* was 1000 $\mu L \cdot L^{-1}$.

Then, they observed in *in vivo* tests that waxes amended with two times this octanal MFC effectively prevented the development of green mold on Satsuma mandarins incubated at 25 °C. They discussed that the coatings increased the activity of the enzymes SOD and catalase (CAT), but reduced the activity of phenylalanine ammonia-lyase (PAL) and POD, preventing the accumulation of H_2O_2. Commercial carnauba and/or shellac coatings amended with *Cinnamomum zeylanicum* essential oil at 0.5% (*v*/*v*) provided very high preventive activity against citrus green and blue molds, with disease reductions of about 90% with respect to uncoated control fruit treated with distilled water [114]. However, when the essential oil from *C. zeylanicum* was incorporated into polyethylene commercial waxes, disease reduction was considerably lower, indicating that the antifungal activity of the coating depended not only on the type and amount of essential oil and the volume of coating that remained on the fruit skin, but also on the formulation solubility and the particular compatibility between the essential oil and components of the wax. Among fifty-nine commercially available essential oils, workers from South Africa [115] selected lemongrass, the essential oil from the plant *Cymbopogon citrates*, as the most cost-effective compound for the control of the pathogen *G. citri-aurantii*, the cause of citrus postharvest sour rot. They developed new coating formulations based on the addition of lemongrass and *M. spicata* essential oil to a citrus commercial wax. The coating containing both essential oils, each at a concentration of 750 $mL \cdot L^{-1}$, effectively controlled sour rot, but also green and blue molds, on artificially-inoculated "Valencia" oranges.

7. Citrus Coatings Formulated with Microbial Antagonists

Research in the 1990s showed that different coatings containing microbial antagonists as biocontrol agents could be of use for the control of postharvest diseases of citrus fruit. Strains of the yeasts *Candida oleophila* ([116–118], *Candida guillermondii* [119,120] and the bacterium *Pseudomonas syringae* [121] were incorporated to different coatings and assessed against green and blue molds on oranges, lemons, or grapefruits. The effectiveness of these coatings greatly depended on their ability to support populations of the biocontrol agent. Shellac and other commercial wax coatings could be toxic to the yeasts due to the addition of alcohols and basis that are used to dissolve the primary constituents. The survival of the antagonistic yeast *C. olephila* in shellac formulations significantly improved in coatings containing less than 6% alcohol and when concentrations of morpholine and ammonia were minimized. These formulations significantly improved shelf life of coated grapefruits when compared to fruit coated alone with a shellac formulation [117].

Coating formulations based on water soluble cellulose derivatives, such as HPMC or methylcellulose, amended with the biocontrol yeast *C. olephila* significantly reduced decay and prolonged the storage life of grapefruits [116]. Yeast populations were stable in the coatings, but the addition of additional food preservatives was necessary to prevent the growth of other contaminating microorganisms. Thus, the addition of 0.15% PS to these formulations controlled microbial development without reducing the population of *C. oleophila*, which continued to increase in coated fruit during a storage period of 5 weeks. Similarly, the use of commercial sucrose ester formulations as coating matrixes favored the development of *C. olephila* populations in a greater extent than shellac formulations and these coatings were more effective for decay control on grapefruits than shellac-based coatings [118]. Varying the cellulose component of the coating formulation also affected the survival of two yeast biocontrol agents, *C. guillermondii* strain US7 and *Debaryomyces* sp. strain 230. Populations of both strains during incubation at room temperatures were higher in methylcellulose matrixes. The application of a methylcellulose-based coating containing the strain US7 to naturally infected “Pineapple” and “Valencia” oranges resulted in significant decay control for the first 2 to 4 weeks of storage at 16 °C and 90% RH [120].

In very recent research, Aloui, *et al.* [122] investigated the properties and the performance of bioactive coatings formulated with matrixes of sodium alginate and locust bean gum containing cells of the killer yeast *Wickerhamomyces anomalus*. They found that the survival of the yeast in these films was very high (more than 85% of the initial population) and their barrier, mechanical, and optical properties were satisfactory. When applied to “Valencia” oranges artificially inoculated with *P. digitatum*, these coatings reduced green mold by more than 70% on fruit incubated at 25 °C. In addition, coating application effectively reduced fruit weight loss and maintained rind firmness with respect to uncoated oranges.

8. Conclusions

Increasing concerns about human health risks and environmental contamination, restricted commercial channels for conventional production, and the proliferation of resistant strains of pathogenic fungi are important problems related to the use of conventional chemical fungicides for postharvest preservation of fresh citrus fruit. These agrochemicals are often applied as ingredients of synthetic waxes, which are needed to extend fruit postharvest life by reducing transpiration and respiration and also to provide gloss and enhance fruit appearance. Cost-effective disease control in the absence of synthetic fungicides requires the implementation of global IDM strategies based, in the postharvest phase, on the adoption of alternative or complementary non-polluting antifungal treatments. Irrespective of their nature, the evaluation of these alternative treatments should generally focus on the control of green and blue molds, caused by the pathogens *P. digitatum* and *P. italicum*, respectively, since they are the most economically important citrus postharvest diseases. Among these alternatives, the development of antifungal edible coatings arises as a new safe technology with potential to overcome both citrus postharvest physiological and pathological problems.

The functionality of chitosan, with an inherent antifungal activity, and composite films and coatings based on polysaccharide- or protein-lipid matrixes can be considerably improved by the incorporation of additional antifungal ingredients such as food additives or GRAS salts, natural compounds like essential oils or other volatiles, and microbial antagonists (yeasts, bacteria) as biocontrol agents. In each particular case, these antifungal agents are typically selected according to their efficacy as stand-alone treatments against target pathogens and, in many cases, their incorporation as ingredients of coatings brings a remarkable improvement of their performance. For example, coating formulations can overcome application problems of effective agents that may induce fruit phytotoxicities or adverse sensory properties when applied alone as aqueous or gaseous treatments. Further, coating application can also increase the agent antifungal activity by regulating its temporal and spatial release or facilitating its continuous and effective contact with the target pathogen.

Due to the high economic value of the worldwide citrus trade, the development of novel antifungal edible coatings for citrus fruit is a very active research field and, as described in the present review, a considerable number of research studies reporting interesting results are available from the specialized literature and will surely increase in the next few years. Despite the substantial progress that has been accomplished in evaluating new antifungal edible coatings, their implementation is still limited, first because of the current availability of highly effective, convenient, and cheaper conventional fungicides, and second because of general limitations associated to the edible nature of food-grade coating components. Low-toxicity and/or persistence, lack of either curative or preventive activity, or excessive specificity are handicaps for commercial application. It should be taken into account, in this sense, that minimal fruit losses due to postharvest decay are required by citrus export markets and, in contrast to that of synthetic chemicals with direct fungicidal activity, the mode of action of antifungal edible coatings is usually rather fungistatic. Consequently, their effectiveness is highly dependent not only on the target pathogen but also on fruit host characteristics such as citrus species and cultivar and fruit physical and physiological condition. Of course, coating performance from the physiological point of view will also be greatly influenced by all these fruit attributes. Therefore, the development of antifungal coatings should be tailored not only to control specific diseases but also for application to specific commercially important cultivars of oranges, mandarins, lemons or grapefruits, according to particular postharvest handling procedures, fruit storage potential, and destination markets. In addition to the development of new coatings formulated with other matrixes and/or antifungal ingredients and the evaluation of new applications for existing promising coatings, future prospects include research to determine potential synergistic effects from the combined application of different types of antifungal edible coatings with other alternative disease control methods (physical, low-toxicity chemical, or biological) in a multifaceted approach within IDM strategies.

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Author Contributions

Lluís Palou drafted and organized this review article. All three authors wrote and revised the review.

Conflicts of Interest

The authors declare no conflict of interest.

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