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GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce

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Abstract

Postharvest decay caused by fungal pathogens is one of the most important factors causing economic losses for the worldwide industry of fresh horticultural produce. Despite the positive results of the use of conventional chemical fungicides, alternatives for decay control are needed because of increasing important concerns related to their massive and continued use. Low-toxicity chemical alternatives evaluated for control of postharvest diseases of temperate, subtropical and tropical fruit, and fruit-like vegetables are reviewed in this chapter. These compounds should have suitable antifungal activity while showing known and very low toxicological effects on mammals and impact on the environment. In addition, they should be exempt from residue tolerances on agricultural commodities. Authorities confirm these characteristics by approving them as food additives or preservatives or as generally regarded as safe (GRAS) substances. Among those of synthetic origin, the most important are inorganic or organic salts, e.g. carbonates, sorbates, benzoates, paraben salts, etc., and composite edible coatings formulated with antifungal ingredients. Hydrocolloids (polysaccharides such as cellulose derivatives, alginates, pectins, or gums, and various plant proteins) and food-grade lipids are the main components of the matrix of composite coatings. Interesting antifungal ingredients include GRAS salts, essential oils, and antagonistic microorganisms. Low-toxicity chemicals of natural origin include plant extracts, including essential oils, antifungal peptides and small proteins, and coatings based on chitosan or plant gels like those from *Aloe* spp. Efficacy and overall performance, advantages, disadvantages, limitations, and potential combined use of these chemical alternatives in hurdle technologies for postharvest decay control are discussed.

Keywords: antifungal peptides, antifungal edible coatings, antimicrobial salts, chitosan, food preservatives, plant extracts

Highlights

- Low-toxicity chemicals alternative to conventional fungicides are reviewed
- They are food additives or GRAS substances from synthetic or natural origin
- GRAS inorganic or organic salts include carbonates, sorbates, benzoates
- Composite edible coatings are formulated with antifungal ingredients
- Chitosan is the most important natural antifungal edible coating
- Other natural compounds include plant extracts, antifungal peptides, and *Aloe* spp. coatings

Content

1. Introduction

Fruit and vegetables are readily consumed in fresh or processed form, directing substantial interest towards maintaining quality of fresh produce. The health and nutritional benefits of fruits and vegetables are significant, due to the presence of large amounts of antioxidants and micronutrients (Ramos et al., 2013). Moreover, these products provide an important source of income and employment in producing countries and are important drivers of their economic development (Mohamed et al., 2011). Thus, a lot of investment is focused on reaching the desirable markets and extending shelf life without compromising the quality of the product. There is, therefore, a huge demand for postharvest technologies for handling fresh produce (Yahia et al., 2011). These technologies should offer protection from postharvest diseases as well as over-ripening. Amongst all pathogens, fungal plant pathogens are more prevalent and are one of the major causes of quality deterioration of fruit and vegetables, mainly due to the high moisture content of these food products. Fungal infections typically results in decay, accelerated ripening, as well as accumulation of mycotoxins (Tripathi and Dubey, 2004). To mitigate the infection pressure and properly control disease, producers rely heavily on application of chemical fungicides. Use of fungicides has led to substantial improvements in maintaining the shelf life of fresh produce. Modern fungicides, however, are organic compounds, with a high degree of specificity towards their target organism. Despite the positive results of the use of modern fungicides, increasing concerns continue to be expressed about health hazards and environmental pollution due to the use of large quantities of chemicals (Bautista-Baños et al., 2006; Gupta and Dikshit, 2010). Furthermore, the continuous use of these synthetic compounds has led in many cases to the proliferation of resistant biotypes of fungal pathogens. The build-up of single, double, and even triple-resistant isolates in the populations of fungal pathogens in commercial packinghouses seriously compromises the effectiveness of these chemicals (Palou et al., 2008). Therefore, new approaches for controlling postharvest diseases have shifted towards prioritizing alternatives to synthetic fungicides.

Among the alternative methods of different nature for decay control, in this chapter we focus on low-toxicity chemicals. Such alternatives to conventional fungicides for postharvest disease control of fresh horticultural produce should be compounds with known and very low toxicological effects on mammals and impact on the environment. According to their origin, these alternatives can be roughly divided into synthetic and natural means. Among the former, the most important are inorganic or organic salts, applied to fruit as aqueous solutions, and synthetic composite edible coatings formulated with antifungal ingredients. The latter can be of plant or animal origin and include plant extracts (including essential oils), antifungal peptides and small proteins, and natural antifungal edible coatings such as chitosan and *Aloe* spp. gels. As food-grade substances, all alternative chemicals should be approved as generally regarded as safe (GRAS) by the United States Food and Drug Administration (US FDA), as food additives by the European Food Safety Authority (EFSA), or as an equivalent status by each competent national legislation. GRAS materials are exempt from residue tolerances on all agricultural commodities by the US FDA. In general, these alternative chemicals can be applied to fresh fruit after harvest as aqueous solutions, vapors, or coating treatments. Since essential oils and other antifungal gaseous compounds of plant origin are primarily applied as fumigants, they are discussed with antifungal volatiles (Mari et al., 2016).

Due to their general low toxicity, application of these chemical alternatives by themselves may not always provide a commercially acceptable level of control of postharvest diseases comparable to that obtained with synthetic fungicides. For this reason, compounds with potential as stand-alone treatments are increasingly being evaluated in combination with other postharvest treatments of the same or different nature as part of 'multiple hurdle' or integrated control strategies. Hurdle technology explores the use of mild treatments that collectively maintain the fruit quality and lower the incidence of postharvest decay. In general, three different objectives may be pursued by the combination of treatments (Palou et al., 2008): additive or synergistic effects to increase the efficacy and/or the persistence of individual treatments, complementary effects to combine preventive and curative modes of action, and commercial application of effective treatments that are too impractical, costly, or risky as single treatments. Clear disadvantages of combined approaches are their higher costs and complexity, which make greater the difficulty to turn their application in commercial practice (Romanazzi et al., 2012). Integration of treatments highlighted in the present chapter are the use of GRAS salts in combination with other alternative control means and combined applications of chitosan, especially with essential oils as additional antifungal agents. Moreover, from a strict point of view, composite edible coatings formulated with antifungal ingredients could also be considered as combined treatments.

2. Inorganic and organic salts

Several inorganic and organic salts, classified as food additives or GRAS substances, have been proved as more or less effective for the control of postharvest diseases of fresh horticultural produce when applied as aqueous solutions after harvest. Most of these substances are listed as food preservatives and have a well-known general antimicrobial activity. Although acidic forms also possess antimicrobial activity in some cases, salts are preferred for postharvest treatments because of their superior solubility, ease of application, and additional activity of cations such as Na^+, K^+, or NH_4^+ (Smilanick et al., 1999).

After being investigated in the early 30s of the last century in California (Barger, 1928), the use of carbonate salts to treat citrus fruits was revisited in the 80s and 90s when problems related to the continued use of conventional synthetic fungicides in citrus packinghouses started to arise. Dip treatments in 2-3% sodium carbonate (SC) or sodium bicarbonate (SBC) aqueous solutions for 60-150 s showed antifungal activity against citrus green and blue molds caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, and their performance was significantly improved by heating the solutions to 45-50 ºC. These treatments were not phytotoxic and also considerably reduced decay on long-term cold stored fruit (Smilanick et al., 1997, 1999; Palou et al., 2001). In general, sodium salts were more effective than other carbonate salts and their antifungal activity was higher on oranges than on mandarins (Palou et al., 2002a). Since then, and after several successful commercial applications attempted in California, SC and SBC have been the most common food preservatives used for decay control in citrus packinghouses worldwide. Advantages are their relative effectiveness, general low cost, and lack of restrictions for many applications including organic agriculture. Many research works have focused on the evaluation of these salts, especially SBC, as one of the components of integrated methods to control citrus postharvest diseases. Besides heating the solutions, postharvest treatments that have been combined with carbonates for this purpose include a number of antagonistic biological control agents, curing, UV-C light, different disinfectants or oxidizers, and conventional fungicides such as imazalil (IMZ), pyrimethanil (PYR), or thiabendazole (TBZ) at low doses (Palou et al., 2008; Dore et al., 2010; Venditti et al., 2010; Cerioni et al., 2012, 2013; Geng et al., 2011; D'Aquino et al., 2013b; Hong et al., 2014). Carbonate salts have also been applied before harvest or in combined applications before and after harvest (Youssef et al., 2012). It was also in the decade of 1980 that the organic salt potassium sorbate (PS), a wide spectrum antimicrobial food preservative, gained research attention for the control of citrus postharvest diseases. Among others, extensive work by Kitagawa and Kawada (1984), Wild (1987), Palou et al. (2002b), Smilanick et al. (2008), Montesinos-Herrero et al. (2009), and D'Aquino et al. (2013a) showed that PS aqueous solutions at 2-3% were effective at different dip conditions, e.g. 2-3 min at room temperature or 30-60 s at 50-62 ºC, against citrus green and blue molds and sour rot caused by the yeast-like fungus *Geotrichum citri-aurantii*. Similarly to carbonates, the effectiveness of these treatments was higher if applied at high temperature and it was clearly influenced by the host species and cultivar, maturity stage, presence of peel wounds and fruit physical condition. Likewise, they were compatible and also synergistic in some cases with low doses of chemical fungicides such as IMZ, TBZ, PYR or fludioxonil (FLU). It was also recently showed that PS dips followed by brief exposures to high CO_2 or O_2 at curing temperature were synergistic for the control of green and blue molds (Montesinos-Herrero and Palou, 2016). PS-treated citrus fruits that will be subjected to prolonged cold storage could be rinsed with tap water at low pressure without reducing considerably the effectiveness of the treatment. It has been claimed that the presence of salt residues on the rind could induce excessive water loss and adversely affect fruit quality (Parra et al., 2014). Other GRAS salts with proven curative activity against citrus green and blue molds include sodium benzoate (SB) (Montesinos-Herrero et al., 2016), sodium parabens (Moscoso-Ramírez et al., 2013), and potassium silicate (PSi) (Moscoso-Ramírez and Palou, 2014). These authors established the best concentration and treatment conditions for dip applications of aqueous solutions of these compounds. All of them were compatible with the fungicide IMZ, their effectiveness was significantly higher on oranges than on mandarins, and can be considered as new tools for potential inclusion in citrus integrated disease management (IDM) programs. PSi, in addition, is included in the list of synthetic substances allowed for use in organic crop production in the USA. A variety of salts have also been evaluated for the control of citrus sour rot (Talibi et al., 2011; Duan et al., 2016).

The use of aqueous solutions of GRAS inorganic and organic salts as antifungal treatments to control major postharvest diseases of stone fruits, i.e. peaches, nectarines, plums, and sweet cherries has been recently reviewed (Usall et al., 2015). Several research works showed that postharvest applications of SBC or potassium bicarbonate (PBC), alone or in combination with other alternative control methods, effectively reduced brown rot caused by different species of *Monilinia* (mainly *M. fructicola*, *M. laxa*, and *M. fructigena*). In other works, heated solutions of PS and SB were the most effective treatments to reduce the incidence of brown rot, gray mold, blue mold, and sour rot, caused by *M. fructicola*, *Botrytis cinerea*, *Penicillium expansum*, and *Geotrichum candidum*, respectively (Palou et al., 2009; Molinu et al., 2012). On table grapes, reduction of the most economically important postharvest disease, gray mold caused by *B. cinerea*, has been accomplished with preharvest and/or postharvest treatments with salts such as SBC, SC, PS, PBC, potassium carbonate (PC), or calcium chloride ($CaCl_2$) (Karabulut et al., 2005; Nigro et al., 2006; Youssef and Roberto, 2014). Due to their higher effectiveness and better integration into usual plant protection practices, field applications, especially of $CaCl_2$, were recommended (Romanazzi et al., 2012). According to common commercial handling in major table grape production areas, postharvest aqueous applications of salts such as SBC or SC would be restricted to the detached berries industry (Mlikota Gabler and Smilanick, 2001). Although not a salt, ethanol is also a GRAS compound with active action against *B. cinerea* that could be of use when sulphur dioxide (SO_2) fumigations or grape packaging with sodium metabisulphite pads are too risky or banned by particular markets (Romanazzi et al., 2012). GRAS salts are also of application on table grapes in combination with other decay control means. For instance, it was found in recent research that SBC enhanced gray mold control and overall fruit quality on grapes treated with the biocontrol agent *Hanseniaspora* *uvarum* (Qin et al., 2015). Similarly, SBC has been frequently evaluated as a component of integrated treatments for the control of the most important postharvest diseases of pome fruits. Thus, improved control of blue mold and black spot of apples or pears, caused by *P. expansum* and *Alternaria alternata,* respectively, has been obtained by combining SBC application with a variety of biocontrol agents, mainly yeasts, and other postharvest treatments such as heat or controlled atmospheres (Yao et al., 2004; Janisiewicz et al., 2008; Janisiewicz and Conway, 2010; Lai et al., 2015). Typically, SBC alone reduced decay in these trials, but was much more effective when combined with the antagonists. In general, SBC or SC are good GRAS candidates for integration with many biological control treatments thanks to the high compatibility that allow the proliferation of microbial antagonists in fruit wounds containing carbonate residues. PS solutions have also been recently tested against blue mold of apples alone or in combination with heat or TBZ (Fadda et al., 2015). An active packaging comprised of polyethylene terephthalate coated with PS was recently developed for gray mold control on raspberries, blackberries, and blueberries (Junqueira-Gonçalves et al., 2016).

Inorganic and organic salts as postharvest antifungal treatments have been also applied to tropical fruits such as avocado, mango, papaya or banana. Avocado production in countries like South Africa, Israel or Chile is export driven, with the European Union (EU) being the biggest market and this entails high fruit quality standards. Among them, the reduction or elimination of chemical residues on/in fruit derived from fungicide use to control major postharvest diseases like anthracnose caused by *Colletotrichum gloeosporioides* is increasingly gaining importance (van Eeden and Korsten, 2013). Silicon (Si) has been reported as a beneficial nutrient, protecting plants against various diseases. Anderson et al. (2005) reported on postharvest Si application to ‘Hass’ avocados intending to reduce occurrence of anthracnose. Postharvest PSi applications had no effect on respiration rate, however fruit firmness, weight loss, mesocarp electrical conductivity (EC), total phenolics concentration, lipid peroxidation as well as polyphenol oxidase (PPO) and catalase (CAT) activity responded positively to the PSi treatments (Tesfay et al., 2011). These workers concluded that silicon might function as a major elicitor increasing free polyphenol concentrations. Results from recent work with mangos indicated that fruit treated with either bentonite or bentonite loaded with PS exhibited reduced decay, delayed postharvest ripening, decreased water loss, maintained high vitamin C levels, preserved titratable acidity (TA), and no changes in flavor (Liu et al., 2014). Preharvest dips of mango fruit in plant defense inducing chemicals integrated with postharvest treatments with inorganic salts and hot water were evaluated for the management of anthracnose on artificially inoculated mango fruit. The application of either salicylic acid or potassium phosphonate at 1000 mg L^{-1} combined with a fruit dip for 3 min in 3% aqueous SBC at 51.5 ºC significantly reduced disease development as compared to other treatments and the control. The treatments also maintained quality of mango, positively affecting pH, soluble solid content (SSC), TA, firmness, and peel color. In contrast to SBC, $CaCl_2$ treatments alone or combined with preharvest plant defense inducers did not significantly reduce anthracnose severity on mango (Dessalegn et al., 2013). The effect of potassium tetraborate on germination of conidia of *C. gloeosporioides*, and postharvest rot of mango were studied. An application of $K_2B_4O_7$ to mango trees at flowering increased fruit set and decreased the incidence of anthracnose on harvested fruit (Shi et al., 2011). In the case of papaya, anthracnose caused by *C. gloeosporioides* is also a major limiting factor in storage and transit that affects all tropical regions where papaya is grown (Hewajulige and Wilson Wijeratnam, 2010). SBC and ammonium carbonate (AC) in paraffin wax-based formulations have been shown to decrease anthracnose in papaya (Gamagae et al., 2004). AC at 3% followed by SBC at 2%, tested alone or in combination with wax, had a positive effect on reducing *C. gloeosporioides* in both naturally and artificially inoculated fruit (Sivakumar et al., 2002). The effects of preharvest $CaCl_2$ application on ripening, activity patterns of pectin modifying enzymes and overall quality of papaya fruit have been investigated (Madani et al., 2014b). Foliar sprays of $CaCl_2$ at 0, 0.5, 1, 1.5, and 2% (w/v) were applied six times during the growing season. The overall quality of treated fruit after 3 weeks at 12 ºC was greater than that of the control. In a different work, Madani et al. (2014a) reported that preharvest application of $CaCl_2$ at 1.5-2% increased calcium content in fruit and significantly reduced anthracnose incidence during 5 weeks of storage at 12 ºC, and delayed initiation of disease symptoms by 4 weeks. Banana fruit are susceptible to several diseases resulting in massive and extensive postharvest losses during transportation and storage. Crown rot, the most important postharvest disease of banana, is a disease complex caused by several fungi including *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Thielaviopsis paradoxa*, and a complex of *Fusarium* spp. (Ranasinghe et al., 2005). Postharvest application of inorganic salts has been evaluated as an alternative to fungicide dips used for commercial management of banana crown rot. Dipping bananas for 10-15 min in SBC, $CaCl_2$, sodium chloride (NaCl), or sodium hypochlorite (NaClO) solutions significantly reduced the incidence of crown rot compared with untreated fruit 17 days after harvest. SC solutions were ineffective (Alvindia et al., 2004). In other work, effective treatments to control crown rot of bananas were SBC and NaClO at 5 g L^{-1} and $CaCl_2$ at 5 g L^{-1} with a surfactant. Some salts, when ameliorated with surfactant, had a phytotoxic effect on banana fruit (Alvindia and Natsuaki, 2007). Natural infections of anthracnose, crown rot, and blossom-end rot were reduced significantly in banana fruit that were treated with 300 mM SBC for 10 min. This treatment followed by dips in a suspension of the bacterial antagonist *Burkholderia spinosa* was also effective. SBC dips increased pH, SSC and thickness of the fruit peel, which could have an indirect or cumulative effect on the reduction of postharvest disease development in bananas (De Costa and Gunawardhana, 2012). The efficacy of SC, SBC, and NaClO, applied alone or in various combinations with another antagonist, *Bacillus amyloliquefaciens* DGA14, was evaluated. The results indicated that the combination of *B. amyloliquefaciens* with 1% SBC managed crown rot disease comparable with synthetic fungicides without negative effects on fruit quality 14 days after treatment (Alvindia, 2013b). In a different approach, salts (SBC, SC, NaClO) were integrated with a hot water treatment (HWT, 50 ºC for 20 min). Postharvest application, involving fruit dipped for 30 min in 1% SC following HWT, and storage for 14 days at 22-25 ºC and 90-95% relative humidity (RH), maintained the overall quality of bananas and reduced the incidence of crown rot disease by 88%, which was comparable with the efficacy of a conventional fungicide treatment. Other treatment combinations such as HWT + NaClO or HWT + SBC were also capable of reducing crown rot, although to a lesser extent. Efficacy of individual treatments, and particularly of HWT alone, was significantly lower (Alvindia, 2013a). In other research, a combination of the fungal biocontrol agent *Trichoderma harzianum* and 1% SBC was the best for crown rot control, with an efficacy similar to synthetic fungicides, and maintained the overall quality of banana even at conditions favorable for the pathogens (22-25 ºC, 90-95% RH) (Alvindia, 2013c).

Some inorganic and organic salts have also been evaluated as antifungal treatments against important postharvest pathogens of vegetables or fruit-like vegetables such as tomato or potato. $CaCl_2$ was tested for the control of postharvest decay development on cherry tomatoes alone or in combination with the marine yeast *Rhodosporidium paludigenum*. It was verified that the combined treatments showed high activity to reduce black rot caused by *A. alternata* (Wang et al., 2010). The inhibitory effect of $CaCl_2$ alone or in combination with cassia oil against this disease was also assessed on cherry tomato. Some combinations showed a significant inhibition effect on decay development in both artificially wounded and unwounded naturally infected fruit. Importantly, these treatments did not reduce the overall quality of tomato. It was observed that the treatments significantly enhanced the activity of some defense-related enzymes such as PPO and peroxidase (POD) (Feng et al., 2013). Mature red cherry tomato fruit were treated with HWT (45 ºC) or 2% SBC, each alone or in combination for 10 min, then stored at 20 ºC for 6 days. HWT alone was not suitable for mature red cherry tomato fruit, while a combination of HWT plus SBC showed potential as a commercial treatment to avoid fruit cracking, improve safety, and maintain fruit quality. Compared with the individual treatments, the combined treatment also reduced the residual procymidone fungicide content of the fruit. After storage, fruit subjected to the combined treatment had higher skin firmness and TA, with a lower incidence of gray mold caused by *B. cinerea* (Shao et al., 2012). On potato, in vitro trials were conducted to evaluate the effect of several organic and inorganic salt compounds at three different concentrations on the development of postharvest pathogens such as *A. alternata*, *B. cinerea*, *Fusarium solani* var. *coeruleum*, *Phytophthora* spp., and *Verticillium* spp. Overall, mycelium growth and spore germination of all pathogens were strongly inhibited by sodium metabisulfite and propyl-paraben. Spore germination in most pathogens was consistently inhibited by various aluminum salts (Mills et al., 2004). In other research, several salts were found to control bacteria such as *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*) and *Pectobacterium atrosepticum* (formerly *Erwinia carotovora* subsp. *atroseptica*), the causal agents of blackleg and tuber soft rot, respectively, that can contaminate potato tubers both in the field and after harvest. Potato tubers treated with SBC, NaClO, aluminum acetate, alum, calcium propionate, or copper sulfate pentahydrate resulted in significantly less soft rot than the untreated control. These compounds demonstrated a potential as replacements for commercially used postharvest pesticides (Mills et al., 2006). In recent work, soft rot of potato tubers was significantly controlled with aluminum chloride ($AlCl_3$) and sodium metabisulfite, and to a lesser extent with SB, PS, SP, and aluminum lactate (Yaganza et al., 2014).

Although the exact mode of action of many GRAS salts on reducing postharvest disease has not been completely explained, there is evidence that their inhibitory ability depends on the presence of salt residues in the infection courts occupied by the pathogen, typically fruit peel wounds, and on interactions between this residue and constituents of the fruit tissues. Among such interactions, particular direct toxic action of the different anions and cations, pH alterations, and indirect factors related to the induction of disease resistance mechanisms on the fruit host (lignification, biosynthesis and/or accumulation of antifungal compounds correlated with the up-regulation of the phenylpropanoid pathway, etc.) have been described (Palou et al., 2001; Venditti et al., 2005; Youssef et al., 2014). The fact that the induction of resistance is dependent on host factors like the genotype (species and cultivar), maturity stage, and physical and physiological condition can explain why these factors also strongly influence the effectiveness of salt treatments (Moscoso-Ramírez et al., 2013). This dependence, the lack of residual effect to protect the fruit against subsequent infections, the risks of adverse effects on fruit quality during long-term storage, and, above all, the lower efficacy and persistence of the treatments are general disadvantages of GRAS salt treatments compared with conventional fungicides. Additional problems are the lack of regulatory approval for many compounds evaluated in the laboratory and disposal issues related to high salinity, sodium content, pH, and conductivity of some salt aqueous solutions (Palou et al., 2008; Smilanick et al., 2008). Besides the residue tolerance that makes them attractive as control alternatives, in some cases even for organic agriculture, general advantages are their significant curative effect, availability at low cost, and especially the high complementarity with other treatments that would allow their use as hurdle technologies in postharvest IDM programs.

3. Plant extracts

Essential oils are the most important compounds among the wide range of natural substances with antifungal properties extracted from plants. They comprise of a combination of volatile secondary metabolites that are bioactive in vapor phase, show direct activity against phytopathogens, and can also enhance the plant defense mechanisms against these microorganisms (Bautista-Baños et al., 2013). Some of the key active ingredients in essential oils, e.g. cinnamaldehyde, citral, eugenol, limonene, or thymol, are safe for human consumption and have a GRAS status (de Aquino et al., 2015). Therefore, they have been extensively investigated for postharvest applications. Since they have been mainly applied in the vapor phase, they are covered together with antifungal volatile compounds (Mari et al., 2016). However, a viable option to overcome important limitations associated with the use of essentials oils, namely induction of strong odors or flavors, phytotoxicity risks, and lack of efficacy in vivo, would be the incorporation of essential oils into edible coatings and this technology will be discussed later in this review.

In this section, we refer to other aqueous or organic solvent extracts of plants or herbs that have been reported as able to reduce decay on harvested horticultural products. Many of these extracts have been obtained from medicinal or exotic plants located in African, Asian, or South American countries. In general, they are biodegradable substances comprised of compounds that exhibit direct fungicidal or fungistatic activity and also some ability to delay ripening and extend the shelf life of treated produce. Typically, these compounds are products of secondary metabolism produced by the plant for its own protection against pests and pathogens. Extracts from plants that presented a significant activity against the most important citrus postharvest pathogens *P. digitatum*, *P. italicum*, or *G. citri-aurantii* include garlic (Obagwu and Korsten, 2003), pomegranate (Li Destri Nicosia et al., 2016), *Sanguisorba* sp., *Orobanche* sp. (Gatto et al., 2011), Huamuchil (Barrera-Necha et al., 2003), *Accacia* sp., *Whitania* sp. (Mekbib et al., 2009), and *Parastrephia* sp. (Ruíz et al., 2016), among many others (Palou et al., 2008; Askarne et al., 2012; Sayago et al., 2012; Talibi et al., 2012). Some of these extracts have also been found to present antifungal activity against pathogens attacking other fresh produce. This is the case of garlic extracts on apples (Daniel et al., 2015) and bananas (Sanwal and Payasi, 2007), or pomegranate extracts on sweet cherries (Li Destri Nicosia et al., 2016), table grapes (Romeo et al., 2015), and potato tubers (Elsherbiny et al., 2016). In the case of stone fruits, plant extracts with activity against *Monilinia* spp. and other postharvest pathogens have been recently listed in a review by Usall et al. (2015). Some extracts from seed kernel of neem plant (*Azadirachta indica*) have been shown to be toxic to various fungal pathogens causing postharvest decay on plums and pears (Wang et al., 2010). A commercial formulation based on extracts from *Abies* spp. was able to control postharvest diseases of strawberries and sweet cherries when applied in the field (Feliziani et al., 2013a; Romanazzi et al., 2013). However, as most of treatments applied on fruit and vegetables, it is important to check the sensorial quality of treated produce, which should be better or at least not change as compared to the common practice. With respect to tropical fruits, plant extracts from the botanical families Sapotaceae (*Achras sapota*, *Chrysophyllum cainito* and *Pouteria sapota*), Caricaceae (*C. papaya*), Fabaceae (*Pachyrrizus erosus*), Leguminosae (*Phythecellobium dulce*), Solanaceae (*Cestrum nocturnum*), and Verbenaceae (*Lantana camara*) showed noteworthy control of various fungal diseases of papaya such as those caused by *C. gloeosporioides*, *Rhizopus* spp., *Aspergillus* spp. and *Mucor* spp. (Bautista-Baños et al., 2013). Likewise, the constituents and secondary metabolites of *Pachyrrizus dulce* and *P. erosus* demonstrated a remarkable control of various postharvest fungi of papaya (Barrera-Necha et al., 2004). Celoto et al. (2011) evaluated the use of methanol and aqueous extracts of *Momordica charantia* applied on banana fruit, observing up to 80% inhibition in the development of lesions caused by *C. musae* when applied 2 days prior to fungus inoculation. In another research with bananas, artificially inoculated fruit dipped in 20% (w/v) extracts of *Acacia albida* and *Prosopis juliflora* at 50 °C showed reduced anthracnose incidence and severity. This combined treatment did not affect the fruit physico-chemical properties and provided the highest percentage of marketable ripe banana fruit (Bazie et al., 2014). Black rot of pineapple caused by the fungus *Chalara paradoxa* was considerably reduced by the application of an extract of *Mormodica charantia* without affecting the postharvest quality of treated fruit (de Souza et al., 2015). Among various plant species tested, aqueous extracts of leaves of papaya and custard apple (*Annona reticulata*) showed important fungistatic effects against *Rhizopus stolonifer* and *C. gloeosporioides* on mango and ciruela (*Spondias purpurea*) during fruit storage (Bautista-Baños et al., 2000, 2003).

Isothiocyanates (ITCs) are extracted from several families of plants that include Brassicaceae, Moringaceae and Resedeaceae (Bautista-Baños et al., 2013). These naturally occurring compounds are the degradation products of glucosinolates and are toxic to many organisms including fungi, bacteria, nematodes, insects and weeds. Their general use against fungal diseases was reviewed by Tiznado-Hernández and Troncoso-Rojas (2006). As antifungal postharvest treatments, they were tested in vivo against pathogens of pome fruits, stone fruits, and strawberries such as *B. cinerea*, *R. stolonifer*, *Mucor piriformis*, *P. expansum*, and *M. laxa* (Mari et al., 1996, 2008; Ugolini et al., 2014), and a method based on the use of allyl-isothiocyanate on citrus fruit was patented in Japan (MITN-C, 2004). They were also applied for inhibition of the growth of *C. gloeosporioides*, *Fusarium oxysporum* and *R. stolonifer*, and for controlling fungal infection of papaya (Ramos-García et al., 2007). Application of phenylethyl ITC was demonstrated to be more effective than the fungicide benomyl. Moreover, Troncoso-Rojas et al. (2005) reported satisfactory control of *A. alternata* in vitro and alternaria rot on bell pepper using 0.03 mg mL^{-1} ITC extracted from cabbage leaves. In another work, benzyl ITC was also effective against alternaria rot on tomato (Troncoso-Rojas et al., 2005). ITCs are natural occurring volatile compounds with a broad spectrum of activity and are safe for human consumption, thus are suitable alternatives for synthetic fungicides.

Propolis is a natural compound made up of esters, carbohydrates, diterpenic acids, and pentacyclic triterpenes. It is used by bees for protecting their hives (Kasote et al., 2015). It is sourced from conifer trees and is characterized with potent antimicrobial and antibiotic activity. Soylu et al. (2008) observed that 70 and 35% ethanol extracts of propolis completely inhibited conidia germination of the citrus postharvest pathogen *P. digitatum*, but failed to control green mold on artificially inoculated grapefruits. Similarly, ethanol extracts of Chinese propolis completely prevented the in vitro mycelial growth of *P. italicum* (Yang et al., 2011). However, no in vivo tests for evaluation of blue mold control were conducted by these workers. Mattiuz et al. (2015) demonstrated the efficacy of propolis in controlling the growth of *C. gloeosporioides* in vitro and reducing anthracnose on mango in vivo. In a study by Ordones et al. (2011), antimicrobial activity of propolis against *Erwinia carotovora* spp. *carotovora*, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas corrugata* and *Xanthomonas campestris* pv. *vesicatoria* was demonstrated at concentrations as low as 9.5 µg mL^{-1}. The authors also demonstrated the activity in controlling *P. syringae* infection of tomato. A major concern with the application of propolis, as with volatile compounds, is the possibility of the treatment causing phytotoxicity on the surface of the fruit in addition to the effect on flavor and aroma if applied at high doses.

4. Antifungal peptides and small proteins

Peptides produced by plants or animals as a defense mechanism against challenging microbes are known, in general, as antimicrobial peptides (AMPs). In parallel to what has occurred in medicine, AMPs and small proteins have been proposed for potential use in agriculture as novel therapeutics for the control of plant diseases. However, limitations of natural peptides such as nonspecific toxicity, low stability, and poor bioavailability led researchers to attempts to artificially synthetize new AMPs with superior properties (Marcos et al., 2008). In this case, production and purification costs are important limitations and alternatives like the use of plants as biofactories through genetic engineering techniques are being increasingly studied (Bundó et al., 2014). 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. For the control of postharvest diseases of horticultural produce, the production of antifungal peptides (e.g. iturins, fengycins, etc.) has been frequently identified as a mode of action of several biological control agents, mainly bacteria, e.g. *Bacillus* spp. (Yánez-Mendizábal et al., 2012; Waewthongrak et al., 2015). Moreover, some plant-produced peptides have shown activity against postharvest pathogens (Alem et al., 2014), and non-natural compounds have been synthetized that also showed promise as effective postharvest treatments. This is the case of the tryptophan-rich, cationic hexapeptide PAF26 and some derivatives, which showed inhibitory activity against citrus pathogens like *P. digitatum* (Muñoz et al., 2007; Harries et al., 2015), or the hybrid undecapeptide BP22, which resulted active against *P. expansum*, the cause of blue mold on pome and stone fruits (Badosa et al., 2009).

5. Antifungal edible coatings

Artificial coating of fresh horticultural produce is common in many packinghouses to reduce weight loss, shrinkage, and improve appearance. Typically, commercial coatings are wax-based compounds, often amended with synthetic fungicides to additionally provide control of postharvest diseases. Currently, there is an increasing interest in the development of antifungal edible coatings of natural (plant or animal) origin or based on biodegradable formulations amended with additional food-grade antifungal compounds in order to replace these commercial waxes. While chitosan-based or *Aloe vera*-based coatings present inherent antimicrobial activity, synthetic biopolymer-based coatings are formulated with antimicrobial ingredients that, according to their nature, can belong to three different categories (Palou et al., 2015): i) synthetic food preservatives or GRAS compounds such as various inorganic and organic salts, ii) natural compounds such us essential oils or other natural plant extracts, and iii) microbial antagonists as biocontrol agents (bacteria, yeast, yeast-like fungi, and even some filamentous fungi, covered in the review by Droby et al. (2016)). As components of edible formulations, compounds in the first two groups should be classified as food-grade additives or GRAS compounds by the regulation agencies.

5.1. Chitosan and derivatives

Chitosan is a natural, biodegradable, biocompatible, and non-toxic biopolymer obtained from chitin deacetylation that is sourced from the exo-skeleton of crustaceans (Muzzarelli and Muzzarelli, 2005; Lizardi-Mendoza et al., 2016). It is a biopolymer that needs to be dissolved in weak acids, in a solution having a pH of at least 2.8 (usually acetic acid at 0.5 or 1%), and its antimicrobial activity and physical properties (e.g. viscosity, coating thickness) varies according to the acid used for dissolution (Romanazzi et al., 2009). There is a high interest for its use in agriculture because when it is applied on plants, it shows a triple action: antimicrobial activity on plant pathogens, film-forming activity that acts as barrier, and ability to elicit plant defense mechanisms, that makes it an ideal coating for fruit and vegetables (Romanazzi et al., 2016). Chitosan possess direct antimicrobial properties and has been proven to control fungi and bacteria in vitro and in vivo, including *C. gloeosporioides*, *R. stolonifer*, *P. digitatum*, and *F. oxysporum* (Bautista-Baños et al., 2013). Growth inhibition induced by chitosan on *P. digitatum*, *P. italicum*, *Botryodiplodia lecanidion*, and *B. cinerea* ranged from 25 to 95% (Chien and Chou, 2006). At 1%, it decreased the radial growth of decay causing fungi like *B. cinerea*, *A. alternata*, *M. laxa* and *R. stolonifer* at the same rate or close to the fungicide fenhexamide (Feliziani et al., 2013a). Moreover, it was found effective in the control of a list of foodborne bacteria, mainly when used as chitosan nanoparticles (Sotelo-Boyás et al., 2016). On the other hand, chitosan produces a film on the treated fruit surface that reduce gas exchange and respiration (El Ghaouth et al., 1991; Romanazzi et al., 2009), then slowing down ripening and keeping the fruit less susceptible to decay. Chitosan hydrochloride was approved in the EU as the first product in the list of basic substances in plant disease management (Reg. EU 563/2004) and some commercial formulations should soon be registered for use as plant protection products. This aspect will increase its use and lower the cost of formulations, so it will be easier for growers to test the feasibility of large scale chitosan applications, both in organic and in conventional agriculture, to improve the management of postharvest decay.

Chitosan and derivatives are currently the most assayed antifungal edible coatings for postharvest preservation of fresh horticultural produce. Chitosan has been applied to prolong storage and shelf life of a long list of temperate fruit, including apple, pear, peach, sweet cherry, strawberry, blueberry, raspberry, and table grapes, among others (Romanazzi et al., 2016). Thirty years ago, Muzzarelli (1986) reported that chitosan had all properties of an ideal biopolymer for coating of fruits and vegetables. The research on the application of chitosan on fruit was started in the last decade of the last century by Ahmed El Ghaouth and coworkers (El Ghaouth et al., 1991, 1992a) in Joseph Arul's laboratory at the University of Laval (Canada). They used chitosan on strawberry fruit kept at 13 °C, finding an extended storage and a reduction of gray mold and Rhizopus rot, with a concurrent direct effect (radial growth inhibition and hyphal deformation) on *B. cinerea* and *R. stolonifer*. In the meantime, they applied chitosan to protect tomatoes, which appears to be the first application on vegetables (El Ghaouth et al., 1992b). Since then, several research groups found interest in the use of chitosan, and further information on its coating properties were observed on strawberries (Zhang and Quantick, 1998; Reddy et al., 2000b), table grapes (Romanazzi et al., 2002; Meng et al., 2008), and sweet cherries (Romanazzi et al., 2003) sprayed in the field with chitosan formulations. Most of the first trials were run with chitosan powder dissolved in weak acids, and in the last years a list of commercial formulations were made available on the market (Elmer and Reglinski, 2006; Romanazzi et al., 2016). Some commercial chitosan formulations had the same effectiveness than practical grade chitosan dissolved in weak acids (Feliziani et al., 2013b, 2015). However, water dissolvable chitosan formulations are much easier to use for growers and have a higher potential interest. One of the most important aspects researchers focused on was the effect of chitosan applications on fruit quality. In trials run on strawberries, chitosan treatments did not affect the taste of the fruit after application, except very soon after application, when a slightly bitter taste was perceived (Devlieghere et al., 2004). In general, in most of the studies, overall fruit quality was not negatively affected by chitosan application (El Ghaouth et al., 1991; Devlieghere et al., 2004; Feliziani et al., 2015). Another positive aspect of chitosan versus synthetic fungicides is its typical persistence on plant tissues. In trials in which it was applied once, 21 days before harvest, or twice, 21 and 5 days before harvest, no differences in decay development on table grapes were observed after 30 days of cold storage and 4 days of shelf life (Romanazzi et al., 2002).

Chitosan has also been evaluated on a variety of citrus and other subtropical and tropical fruits. Significant reductions of citrus green or blue molds were obtained in laboratory trials with oranges, lemons, mandarins, or grapefruits artificially inoculated with *P. digitatum* or *P. italicum* and treated with chitosan or derivatives such as glycol chitosan (El Ghaouth et al., 2000; Chien et al., 2007; Zeng et al., 2010; Panebianco et al., 2014). Both direct and indirect effects (enhanced activity of enzymes such as POD or superoxide dismutase (SOD)) for disease reduction were reported in these studies. Furthermore, oligochitosan, another hydrolyzed derivative, was effective to reduce anthracnose caused by *C. gloeosporioides* on oranges (Deng et al., 2015). On papaya fruit treated with chitosan and stored at room temperature, 150 kDa chitosan successfully controlled mesophilic bacteria, yeast, and molds, extending the shelf life of the fruit for 4 to 7 days (Dotto et al., 2015). Hong et al. (2012) reported that application of 2% chitosan coating on ‘pearl’ guava stored at 11 °C for 12 days significantly enhanced the antioxidant ability of the fruit, which significantly delayed fruit ripening.

While studies on the application of chitosan on fruit is popular, at least at research stage, the use of chitosan to manage postharvest decay of vegetables is not deeply studied as well. After first investigations by El Ghaouth et al. (1992b) on tomatoes, it was later applied on the same crop with positive results in terms of decay management and fruit quality maintenance (Reddy et al., 2000a; Liu et al., 2007; Badawy and Rabea, 2009). The application of chitosan on vegetables as asparagus, broccoli, carrot, green beans, potato, radish, red bell pepper, squash, and sweet pepper was recently reviewed by Miranda-Castro (2016).

Nanotechnology applied for development of innovative chitosan-based coatings has been a new direction explored in recent years. Chitosan nanoformulations are characterized by the smaller size, which allows for encapsulation of the functional ingredients and reduced chemical degradation (Mustafa et al., 2013). Ing et al. (2012) demonstrated the antimicrobial activity of chitosan in vitro against the pathogens *Fusarium solani* and *Aspergillus niger*, which was more effective when the high molecular weight chitosan was used to prepare the nanoformulation. Applications of chitosan nanoformulations at 1, 1.5, or 2% were similarly effective in controlling the growth of *Colletotrichum musae* and *C. gloeosporioides* (Zahid et al., 2012). The authors also demonstrated the effectiveness of the chitosan nanoformulation at concentration of 1% in controlling anthracnose of banana, papaya, and dragonfruit. The use of nanoformulation allowed the authors to apply lower doses of chitosan (1%) than would normally be applied (1.5 or 2%). Nanotechnology was also explored by Jiang et al. (2013) in the application of alginate/nano-Ag coatings for reducing the microbial load and extending the shelf life of shiitake mushrooms.

5.2. Combined applications of chitosan

Chitosan has the properties to be applied alone, but it works well when combined with other alternatives to synthetic fungicides in order to exploit additive or synergistic effects. Although chitosan alone shows antimicrobial activity at standard doses (usually 1%), reducing the rates is compatible with the application of other disease control methods of different nature such as inorganic and organic salts, ethanol, UV-C irradiation, plant extracts, essential oils, biocontrol agents, modified active packaging, or hypobaric treatment. Further, chitosan is also compatible with the addition of vitamins, ascorbic acid, or other bioactive compounds that can enhance fruit quality (Romanazzi et al., 2012, 2016).

Early work by El Ghaouth et al. (2000) showed that glycol chitosan, a water dissolvable chemical formulation of chitosan, combined with SC and a biocontrol yeast improved the control of postharvest decay of apple and citrus fruit. Sivakumar et al. (2005) reported that chitosan alone or in combination with SBC or AC significantly reduced the severity of anthracnose in papaya fruit. The effect of chitosan with AC on the incidence and severity of anthracnose was greater than chitosan alone, or chitosan with SBC. Eating quality was not affected by these postharvest dip treatments. A combination of chitosan with AC retained high fruit quality, significantly retarded color development of skin and flesh, increased fruit firmness and reduced weight loss. Al Eryani-Raqeeb et al. (2009) demonstrated the efficacy of calcium (2.5%) and chitosan (0.75%) infiltration in controlling *C. gloeosporioides*. The development of this fungus on fruit treated with this combination was seriously affected since no conidial germination took place and the incidence of anthracnose was 38%

compared with the 88% shown in the untreated fruit. Additional advantages of this treatment were an extended storage life of approximately 15 days and reduced weight and firmness loss.

There is no doubt the most frequent combined application of chitosan is the incorporation of plant extracts, essential oils or their components into chitosan coating matrixes. In general, this allows a unique postharvest treatment with additional properties for fungal growth inhibition and fruit quality maintenance (Palou et al., 2015). Chitosan combined with citral or lemongrass oil significantly controlled green and blue molds and sour rot on artificially inoculated oranges and limes (Faten, 2010; El-Mohamedy et al., 2015). Chitosan coatings amended with essential oils from bergamot, thyme, or tea tree were more effective than chitosan alone in reducing blue mold of oranges caused by *P. italicum*, providing both preventive and curative activity (Cháfer et al., 2012). It was concluded in another work that chitosan improved the release of Mexican oregano, cinnamon, or lemongrass essential oils to inhibit the pathogens *P. digitatum* and *A. niger* (Avila-Sosa et al., 2012). Nevertheless, in a recent study by Shao et al. (2015) the combination of chitosan with clove oil for inhibiting *P. digitatum* on citrus fruit did not demonstrate enhanced activity in comparison with treatment with chitosan alone, despite that the combination of chitosan and clove oil enhanced the activity of some fruit defense enzymes. Working with guava fruit, de Aquino et al. (2015) explored the activity of chitosan-cassava starch coating in combination with the essential oils extracted from *Lippia gracilis*, which contained mostly thymol and carvacrol. Combination of the three constituents at 2% chitosan, 2% cassava and 1, 2 and 3% essential oil were effective in controlling microbial infection of the fruit. Mattiuz et al. (2015) reported that the performance of propolis on mango in vivo for the control of anthracnose and maintenance of fruit quality was better for fruit treated with 1.5% chitosan. Barrera et al. (2015) explored propolis at 5% in combination with 1% chitosan for controlling anthracnose of papaya and reported reduced fruit decay and infection diameter of the causal agent of anthracnose in papaya, *C. gloeosporioides*. Bautista-Baños et al. (2003) found that the combination of 2.5% chitosan with aqueous extracts of custard apple leaves, papaya leaves, or papaya seeds had a fungistatic rather than fungicidal effect on the development of *C. gloeosporioides*. Chitosan applications did not influence SSC or weight loss during the storage of papaya fruit. However, there was a tendency toward greater firmness in fruit treated with the papaya seed extract alone or combined with chitosan. On vegetables, the combined application of chitosan and cinnamon oil improved decay control of sweet pepper (Xing et al., 2011), while when it was combined with natamycin, it reduced *Fusarium* and *Alternaria* rots of melon (Cong et al., 2007). In general, the activity of essential oils is attributed to the presence of active ingredients, characterized with both antimicrobial and antioxidant properties (Sivakumar and Bautista-Baños, 2014). The activity of chitosan plus essential oils operates either through direct effect on the pathogen or through enhanced plant defense mechanisms such as phenylalanine ammonia-lyase (PAL), chitinase (CHI), and ß-1,3 glucanase activity (Zhang et al., 2011).

Chitosan has been recently explored in combination with plant growth regulators for enhanced control of fruit shelf life. A chitosan-g-salicylic acid complex was found to maintain the quality attributes of cucumber, while inhibiting chilling injury (Zhang et al., 2015). Moreover, antioxidant activity of the fruit (SOD, APX and CAT), as well as endogenous salicylic acid content, was enhanced by this treatment, which directly enhanced the defense mechanisms and quality attributes of the vegetable. Kumari et al. (2015) also explored the combination of chitosan and salicylic acid on litchi fruit stored at 4 °C. The treatment was found to delay decay loss of the fruit, while maintaining the fruit quality and nutraceutical content, reducing susceptibility to disease. Qiuping and Wenshui (2007) treated Indian jujube fruit with the ethylene inhibitor 1-methylcyclopropene (1-MCP) and then coated the fruit with chitosan. The combination of the treatments was effective in extending the shelf life of the fruit by a total of 8 days and enhancing the bioactive constituents such as ascorbic acid content, which are important in natural defense mechanisms.

While chitosan is a strong antifungal agent, it can block fruit respiration when applied at high concentrations. A novel application of chitosan is the development of bilayer coatings, which have the capacity to provide a uniform matrix that delivers the desirable properties of chitosan without deteriorating the fruit quality. Arnon et al. (2014) applied a bilayer coating comprising of chitosan and carboxymethyl cellulose (CMC) as a treatment for citrus fruit. They reported enhanced firmness retention, which would greatly reduce the susceptibility of the fruit to infection by phytopathogens. Mustafa et al. (2013) and Ali et al. (2014) reported a better delivery of chitosan when applied as nanoformulation into the fruit, but they also observed poorer permeability properties for chitosan. Thus, Ali et al. (2014) explored the benefits of bilayer coatings by coating fruits with a layer of 600 nm chitosan nanoemulsion, followed by a layer of 1% conventional chitosan coating for maintaining the quality of dragon fruit during storage for 28 days.

5.3. Aloe *spp. coatings*

Gels and aqueous extracts 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 in different industries (medicinal, pharmaceutical, cosmetic, tonic drinks, and others in the food industry), including the treatment of horticultural products. The gels have a complex chemical composition, mainly polysaccharides and soluble sugars, followed by proteins, many of which are enzymes, aminoacids, vitamins, and anthraquinones

(Zapata et al., 2013). Similarly to chitosan, the physical and chemical properties of the gels allow their use as edible coatings for physiologic preservation of fruits and vegetables, but they have been less studied as antifungal treatments. Early work by Saks and Barkai-Golan (1995) showed significant activity of *A. vera* gels against *P. digitatum* and against green mold on grapefruits artificially inoculated with this fungus. Recently, Jhalegar et al. (2014) found that an *A. vera*-based coating reduced green and blue molds on mandarins. Different research showed the in vitro inhibitory activity of *A. vera* gels against the most important postharvest pathogens of stone fruits and table grapes, and also the high antifungal action of preharvest or postharvest coating applications of these gels on peaches, plums (Guillén et al., 2013), nectarines (Navarro et al., 2011), sweet cherries (Martínez-Romero et al., 2006), table grapes (Valverde et al., 2005; Castillo et al., 2010), strawberries (Sogvar et al., 2016), or avocados (Bill et al., 2014). Most of these studies also demonstrated the good performance of these coatings to delay ripening and preserve functional properties and overall quality of treated fruit. In addition, the activity of *A. vera* coatings has been reinforced in some cases through the incorporation of additional ingredients like essentials oils or acetic acid (Bill et al., 2014; Paladines et al., 2014; Sogvar et al., 2016).

5.4. Composite antifungal edible coatings

Substantial research has been devoted in recent years to the development of novel synthetic, food-grade composite coatings with antimicrobial properties. The term composite indicates that the matrix of the coating contains a combination of hydrocolloids (polysaccharides or proteins) with lipids (waxes, acylglycerols, or fatty acids). In general, hydrocolloids provide good gas barrier characteristics, but poor water barrier characteristics due to their hydrophilic character. Conversely, lipids, as hydrophobic compounds, provide an appropriate barrier to moisture and also gloss to enhance the appearance of coated produce. These are the basic components, but plasticizers and emulsifiers or surfactants are often also added as matrix components to improve different characteristics of the emulsion. These matrixes may be directly used as coatings or be carriers of additional ingredients added to widen the emulsion functionality (Han, 2014). In the particular case of antifungal edible coatings, the additional ingredients are food-grade compounds of different nature with proven antifungal properties (Valencia-Chamorro et al., 2011).

Incorporating essential oils or plant extracts into composite edible coatings can be a viable option for addressing some of the problems that have been witnessed when applying essential oils for delaying disease symptoms of fresh fruit and vegetables. This allows the control of the pathogens, while regulating the diffusion process of the volatile constituents of the essential oil (Shao et al., 2015). Moreover, essential oils have been incorporated into edible coatings for enhancing the coating properties. They have the potential to extend the shelf life of the produce, maintaining the desirable quality attributes of the produce (Sung et al., 2013). The combination of numerous elements can prove beneficial for the quality and shelf life of the plant product. Edible coatings have been developed, which explore the combination of essential oils with polysaccharide and lipid based composite coatings. On citrus fruits, although research has focused mainly on the incorporation of essential oils into commercial waxes (not edible), few works investigated the addition of natural antifungal plant compounds to edible hydrocolloid-lipid formulations. Thus, hydroxipropylmethyl cellulose (HPMC)-lipid films formulated with different concentrations of an ethanolic extract of propolis effectively inhibited the pathogen *P. italicum* in in vitro tests (Pastor et al., 2010). Carboxymethyl cellulose (CMC) coatings containing essential oil from *Impatiens balsamina* significantly reduced natural decay on long-term stored oranges (Zeng et al., 2013). Similarly, *Penicillia* molds were significantly controlled on artificially inoculated oranges treated with a pectin-based edible coating amended with essential oil at different concentrations (Velásquez et al., 2014). The use of pullulan coating enriched with *Satureja hortensis* extracts on pepper and apple decreased weight loss and improved fruit quality parameters during 2-4 weeks of storage, together with an antimicrobial activity against some foodborne bacteria (Krasniewska et al., 2014). Working with tropical fruits, Bósquez-Molina et al. (2010) investigated the role of thyme essential oil in combination with mesquite gum-based and with candelilla wax on papaya, and reported 40% reduction in decay caused by *R. stolonifer* and 100% reduction in decay caused by *C. gloeosporioides*. The combination of 0.4% cinnamon essential oil with 10% gum Arabic was explored by Maqbool et al. (2011) for controlling anthracnose of banana and papaya. The authors reported 80% and 71% lower incidence of decay caused by *C. musae* and *C. gloeosporioides*, respectively. The activity was ascribed to the presence of the essential oils, since gum Arabic alone did not exhibit antimicrobial properties.

Another group of antifungal compounds that have been explored as ingredients of edible composite coatings is comprised of synthetic food additives or GRAS substances such as some inorganic and organic acids and their salts. In extensive work with citrus fruits conducted in Valencia (Spain), a variety of HPMC-beeswax edible composite films containing PS, SB, SP, SMP, and some mixtures of these preservatives as the most effective salts, was developed and tested on commercially important orange and mandarin cultivars for the control of green and blue molds (Valencia-Chamorro et al., 2008, 2009, 2011). The efficacy and overall performance of the coatings was strongly dependent on the susceptibility of each citrus cultivar to decay caused by *Penicillium* spp., and it was generally higher on oranges than on mandarins. This result could be ascribed to a thinner and less reactive skin of mandarins as compared to oranges. In general, the coatings reduced fruit weight loss and maintained firmness without adverse effects on the overall sensory quality of coated fruit. Although not covered in this review, commercial citrus waxes amended with GRAS salts instead of conventional fungicides have also been evaluated against citrus postharvest diseases. Among a number of common preservative salts, PS, AC, ABC, and paraben salts incorporated into HPMC-lipid coatings showed the best performance to control brown rot caused by *M. fructicola* and preserve postharvest quality of plums (Karaca et al., 2014). Polysaccharide edible coatings based on guar gum or pea starch formulated with PS significantly reduced decay caused by several postharvest pathogens on apples, cucumbers, and tomatoes (Mehyar et al., 2011). Fagundes et al. (2013) reported that HPMC-lipid materials formulated with antifungal GRAS salts controlled black spot of cherry tomato caused by *A. alternata* more effectively than gray mold caused by *B. cinerea*. The best results for reduction of gray mold were obtained with materials containing 2% PC, PBC, AC, or ammonium phosphate (AP), while 2% sodium paraben salts were the best ingredients for coatings against black rot. Food preservatives selected from previous research included PC, AP, AC, and sodium propionate (SP) (Fagundes et al., 2014). All antifungal compounds significantly reduced gray mold development on inoculated and cold-stored cherry tomatoes, the SP-based compound being the most effective. The AC-based compound was the most effective to control weight loss and maintain the firmness of coated fruit. Respiration rate, firmness, color, sensory flavor, off-flavor, and fruit appearance were not adversely affected by the application of the antifungal coatings.

6. Concluding remarks

Due to the high economic value of worldwide trade of fresh horticultural produce and the important problems related to conventional fungicides, the development of novel and environmentally-friendly physical, biological, and chemical methods for postharvest disease control is a very active research field on many public and private institutions all over the world. As highlighted in the present review, a considerable number of investigations describing new low-toxicity chemical methods and reporting interesting results for decay control is available from the specialized literature and will certainly increase in the next few years. GRAS salts, plant extracts, peptides, and natural and synthetic edible coatings with proven antifungal properties have been evaluated against many important postharvest pathogens of temperate, subtropical and tropical fruit, and fruit-like vegetables. With this intensive research work, the possibility of identifying new potent antifungal compounds and developing suitable non-polluting chemical alternatives for commercial marketing appears to be bright.

Despite this substantial progress, the general commercial implementation of alternative chemicals 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 low toxicity and edible nature of these alternatives that make unrealistic to assume that they have the same fungicidal activity as conventional fungicides. Key limitations identified with the use of various forms of GRAS or natural compounds include limited curative or preventive activity and persistence and too narrow spectrum of action. Inconsistent results are often reported, that differ depending on the type of crop or nature of disease or storage conditions. This has wide implications in terms of commercializing such technologies, as it is apparent that most applications need to be tailor-made for specific plant products. It should be taken into account, in this sense, that fruit export markets require minimal disease incidence and, in contrast to conventional chemical fungicides that directly kill the target pathogen, alternative means have often a rather fungistatic mode of action. Thus, their effectiveness is also highly dependent on fruit host characteristics such as species and cultivar and fruit physical and physiological condition at the time of treatment, particularly peel condition and ripening stage. Moreover, some natural compounds like essential oils or other plant extracts with potent activity, often may have a negative impact on the flavor and aroma of treated fruit or may even result in phytotoxicity, being the range among effectiveness on decay-causing fungi and lack of phytotoxic effects often very limited or absent. Additionally, while in vitro studies tend to be positive, it is not always the case with in vivo application of the natural compounds, as the treatment may affect the fruit physiology and often may need a suitable carrier for efficient application on the fruit surface.

Although more complex and possibly more expensive, the best strategy for overcome these limitations is, besides the continuous search for new effective single compounds, the integration with other low-risk treatments to optimize disease control efficacy and general performance taking advantage of additive or synergistic effects. The formulation of chitosan coatings or synthetic composite edible coatings with the addition of essential oils or other GRAS compounds may represent an effective way to, besides improving the global functionality of the coating, reduce the risks of induction of adverse sensory properties or phytotoxicity associated with the use of these ingredients as stand-alone gaseous or aqueous treatments. Furthermore, coating application can also increase the antifungal activity of the ingredient by regulating its temporal and spatial release or facilitating its continuous and effective contact with the target pathogen. The integrated approach, however, should not be limited to combinations of postharvest treatments. Cost-effective postharvest disease control in the absence of conventional fungicides requires the implementation of global IDM programs that take into account all preharvest, harvest, and postharvest factors that may influence disease incidence. The purpose of these programs, based on comprehensive knowledge of the aspects defining the disease triangle, i.e. pathogen, fruit host, and environment, is to define all the actions needed during the entire fruit production cycle to minimize final economic losses due to decay. In the development of these multifaceted strategies, emphasis should be placed on minimizing human health risks and environmental toxicity.

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