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 concerns related to their widespread 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. These compounds should have acceptable antifungal activity with known and very low toxicological effects on mammals and minimal 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 these, 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 for edible coatings include GRAS salts, essential oils, and antagonistic microorganisms. Low-toxicity chemicals of natural origin include plant extracts and 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 combinations of these alternatives in hurdle technologies for postharvest decay control are discussed.

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

1. Introduction

Fruits 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 profitable markets and extending shelf life without compromising the quality of the product. There is, therefore, a huge demand for postharvest technologies for handling and maintaining quality of fresh produce (Yahia et al., 2011). These technologies should offer protection from postharvest diseases and physiological disorders as well as delay senescence. Amongst all pathogens, fungal plant pathogens are more prevalent and are major causes of quality deterioration of fruit and vegetables. Fungal infections typically result in decay, accelerated ripening, and in some cases an accumulation of mycotoxins (Tripathi and Dubey, 2004). To mitigate the infection pressure and properly control disease, producers of many important fruit crops rely heavily on application of conventional fungicides. Use of synthetic fungicides has led to substantial improvements in prolonging the shelf life of fresh produce. Modern fungicides are organic compounds, with a high degree of specificity towards their target organism. However, increasing concerns continue to be expressed about health hazards and environmental pollution due to the use of large quantities of chemicals (Gupta and Dikshit, 2010). Furthermore, the continued 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 against several fungicides 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, we focus in this review on low-toxicity chemicals. Such alternatives to conventional fungicides for postharvest disease control of fresh horticultural produce should be compounds with known and minimal 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 substances that will be in contact with fresh produce, all alternative chemicals should be affirmed 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 national legislations. 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 in the review devoted to 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 conventional 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): i) additive or synergistic effects to increase the efficacy and/or the persistence of individual treatments, ii) complementary effects to combine preventive and curative modes of action, and iii) commercial application of effective treatments that are too impractical, costly, or risky as single treatments. Disadvantages of combined approaches are their higher costs and complexity and risks of product injury, which increase the difficulty to turn their application in commercial practice (Romanazzi et al., 2012). Integration of treatments highlighted in the present review 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, 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, were reported as effective to some extent in controlling 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).

2.1. Use on citrus fruits

After being investigated in the early 20s 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 arose. 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., 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., 2002). 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 studies report 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) at low doses (Palou et al., 2008; Dore et al., 2010; Venditti et al., 2010; Cerioni et al., 2013; 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 Wild (1987), Smilanick et al. (2008), Montesinos-Herrero et al. (2009), and D'Aquino et al. (2013) showed that PS aqueous solutions at 2-3% were effective with different dip conditions, e.g. 2-3 min at room temperature or 30-60 s at 50-62 ºC, to control 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, pyrimethanil (PYR), thiabendazole (TBZ), 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).

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).

2.2. Use on temperate fruits

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 reports indicated 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 effective against *B. cinerea* and could be of use when sulphur dioxide (SO_2) fumigations or grape packaging with sodium metabisulphite pads are too risky or banned for particular markets (Romanazzi et al., 2012). GRAS salts can also be used 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 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 due to their 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).

2.3. Use on tropical fruits

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 control of major postharvest diseases like anthracnose caused by *Colletotrichum gloeosporioides* without the use of conventional fungicides is increasingly gaining importance (van Eeden and Korsten, 2013). In this sense, preharvest application to 'Hass' avocado trees of soluble silicon (Si) as trunk injections resulted in a significant reduction of the occurrence of anthracnose (Anderson et al., 2005).

Results from a work undertaken 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 flavor was unchanged (Liu et al., 2014). Preharvest treatment of mango fruit with 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, regarding 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).

In the case of papaya, SBC and ammonium carbonate (AC) in paraffin wax-based formulations have been shown to decrease anthracnose caused by *C. gloeosporioides* (Gamagae et al., 2004). AC at 3% followed by SBC at 2%, tested alone or in combination with wax, significantly reduced *C. gloeosporioides* in both naturally and artificially inoculated fruit (Sivakumar et al., 2002). In other work, Madani et al. (2014) 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.

Postharvest application of inorganic salts has been evaluated as an alternative to fungicide dips used for commercial management of banana crown rot, a disease complex caused by several fungi including *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Thielaviopsis paradoxa*, and a complex of *Fusarium* spp. (Ranasinghe et al., 2005). 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} ameliorated with a surfactant, although in some cases the treatment was phytotoxic (Alvindia and Natsuaki, 2007). Natural infections of anthracnose, crown rot, and blossom-end rot were reduced significantly in banana fruit 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). SBC at 1% in combination with another antagonist, *Bacillus amyloliquefaciens* DGA14, managed crown rot disease comparable with synthetic fungicides without negative effects on fruit quality (Alvindia, 2013b). In a different approach, postharvest application of 1% SC followed by a hot water treatment (HWT, 50 ºC for 20 min) and storage for 14 days at 22-25 ºC and 90-95% relative humidity (RH) reduced the incidence of crown rot disease by 88% while maintaining the overall fruit quality. Efficacy of individual treatments, and particularly of HWT alone, was significantly lower (Alvindia, 2013a). In other work, a combination of the fungal biocontrol agent *Trichoderma harzianum* and 1% SBC was the best for crown rot control, with an efficacy similar to conventional fungicides (Alvindia, 2013c).

2.4. Use on vegetables

Some inorganic and organic salts have also been evaluated as antifungal treatments against important postharvest pathogens of fruit-like vegetables such as tomato or potato tubers. It was found that the combined treatments of $CaCl_2$ with the marine yeast *Rhodosporidium paludigenum* effectively reduced black rot of cherry tomatoes caused by *A. alternata* (Wang et al., 2010). In other work, some combinations of $CaCl_2$ with cassia oil showed a significant reduction of this disease in both artificially wounded and unwounded naturally infected cherry tomatoes, with no adverse effects on fruit quality. It was observed that the treatments significantly enhanced the activity of some defense-related enzymes such as polyphenol oxidase (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 fruit residual content of the botrycide fungicide procymidone. After storage, fruit subjected to the combined treatment had firmer skin, higher TA, and 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. In most cases, spore germination was consistently inhibited by various aluminum salts (Mills et al., 2004). 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).

2.5. Mode of action of salts

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; Dore et al., 2010; 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 (Usall et al., 2008). Additional problems are the lack of regulatory approval for many compounds evaluated in the laboratory and disposal issues related to high salt content, particularly sodium salts, pH, and conductivity of some GRAS 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 and their use will be covered in another article in this special issue (Mari et al., 2016).

Here we discuss 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 originated from 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. (Di Venere et al., 2016), Huamuchil (Barrera-Necha et al., 2003), *Acacia* sp., *Whitania* sp. (Mekbib et al., 2009), and *Parastrephia* sp. (Ruíz et al., 2016), among many others (Palou et al., 2008; Sayago et al., 2012; Talibi et al., 2012). Some of these extracts also have antifungal activity against pathogens attacking other fresh produce. This was 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 were 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 sweet cherries when applied in the field (Feliziani et al., 2013a). 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 changed 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 (*Carica 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 fungal inoculation. In other work 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. ITCs are safe for human consumption, thus are suitable alternatives to synthetic fungicides. 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). Moreover, Troncoso-Rojas et al. (2005) reported that benzyl ITC was also effective against Alternaria black rot on tomato.

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. 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* (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 conventional fungicides to 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). 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. Antagonists are covered in the review by Droby et al. (2016).

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 (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) vary according to the acid used for dissolution (Romanazzi et al., 2009). There is considerable interest for its use in agriculture because its triple action on plants makes it an ideal coating for fruits and vegetables: antimicrobial activity on plant pathogens, film-forming activity that acts as barrier, and ability to elicit plant defense mechanisms (Romanazzi et al., 2016). Chitosan possess direct antimicrobial properties and has been shown 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, when used as chitosan nanoparticles it was found effective in the control of a list of pathogenic fungi and bacteria (Sotelo-Boyás et al., 2016). On the other hand, chitosan produces a film on the treated fruit surface that reduces gas exchange and respiration (El Ghaouth et al., 1991; Romanazzi et al., 2009), thus slowing down ripening and reducing fruit susceptibility 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 it extended storage and reduced gray mold and Rhizopus rot, with a concurrent direct effect (radial growth inhibition and hyphal deformation) on *B. cinerea* and *R. stolonifer*. They also 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), 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 conventional 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). 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).

While studies on the application of chitosan on fruit are 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., 2000; 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 particle size, which allows for encapsulation of functional ingredients and reduced chemical degradation (Mustafa et al., 2013). Ing et al. (2012) demonstrated the antimicrobial activity in vitro of chitosan nanoformulations against the pathogens *Fusarium solani* and *Aspergillus niger*, which was more effective when high molecular weight chitosan was used to prepare the nanoformulation. Applications of chitosan nanoformulations at 1, 1.5, or 2% similarly inhibited the growth of *Colletotrichum musae* and *C. gloeosporioides*, and at 1% effectively controlled anthracnose of banana, papaya, and dragonfruit (Zahid et al., 2012). The effectiveness of the lowest dose of 1% was attributed by the authors to the use of nanoformulations.

5.2. Combined applications of chitosan

Chitosan has the properties to be applied alone, but it works well when combined with other alternatives to conventional 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 means 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 soluble 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 incidence and severity of anthracnose caused by *C. gloeosporioides* on papaya fruit, being the combination with AC the most effective treatment. This combination retained high fruit quality without adverse effects on eating sensory attributes. Al Eryani-Raqeeb et al. (2009) demonstrated the efficacy of calcium (2.5%) and chitosan (0.75%) infiltration in controlling anthracnose of papaya. No conidial germination took place on treated fruit and disease incidence was 38% compared with 88% on untreated control 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). Conversely, 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 in vivo for the control of anthracnose on mango 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 the control of anthracnose of papaya and reported reduced disease incidence and severity. 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*. On vegetables, the combined application of chitosan and cinnamon oil improved control of decay of sweet pepper (Xing et al., 2011), while when it was combined with natamycin, it reduced rots of melon caused by *Fusarium* sp. and *Alternaria* sp. (Cong et al., 2007). In general, the activity of chitosan plus essential oils operates through either direct effect on the pathogen or enhanced plant defense mechanisms such as increased activity of phenylalanine ammonia-lyase (PAL), chitinase (CHI), and ß-1,3 glucanase (Zhang et al., 2011).

Chitosan has been recently explored in combination with plant growth regulators for prolonging fruit shelf life. A chitosan-g-salicylic acid complex was found to enhance the antioxidant activity (catalase (CAT), SOD, and APX), as well as the endogenous salicylic acid content on cucumber, which directly improved the defense mechanisms and quality attributes of the vegetable (Zhang et al., 2015). 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 postharvest decay, while maintaining the fruit quality and nutraceutical content, reducing susceptibility to disease. Qiuping and Wenshui (2007) reported extended shelf life and enhanced bioactive constituents on Indian jujube fruit treated with the ethylene inhibitor 1-methylcyclopropene (1-MCP) and then coated with chitosan.

While chitosan is a strong antifungal agent, it can retard gas exchange and interfere 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 fruit quality. Arnon et al. (2014) developed a bilayer coating comprising of chitosan and carboxymethyl cellulose (CMC) and reported enhanced fruit firmness retention and reduced decay susceptibility when applied to citrus fruit. Ali et al. (2014) explored the benefits of bilayer coatings in increasing permeability properties by coating dragon fruit with a layer of 600 nm chitosan nanoemulsion followed by a layer of 1% conventional chitosan coating.

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). Similar 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. Other 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 several species of stone fruits (Martínez-Romero et al., 2006; Guillén et al., 2013), table grapes (Valverde et al., 2005), strawberries (Sogvar et al., 2016), and 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 observed when applying essential oils to fresh fruits and vegetables for disease control, i.e. induction of strong odors or flavors, phytotoxicity risks, and lack of efficacy in vivo. The incorporation can allow the regulation of the diffusion process of the volatile constituents of the essential oil and also an enhancement of the coating properties (Shao et al., 2015). Edible coatings have been developed that 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 not edible commercial waxes, few 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 *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, Penicillium 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). Working with tropical fruits, Bósquez-Molina et al. (2010) investigated on papaya the role of thyme essential oil incorporated into a coating formulated with mesquite gum and candelilla wax, and reported 40% reduction in decay caused by *R. stolonifer* and 100% reduction in decay caused by *C. gloeosporioides*. Maqbool et al. (2011) reported 80 and 71% lower incidence of anthracnose caused by *C. musae* and *C. gloeosporioides* on banana and papaya, respectively, treated with 0.4% cinnamon essential oil with 10% gum Arabic. The activity was ascribed to the presence of the essential oil, 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. 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. 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 within many public and private institutions worldwide. As highlighted in the present review, a considerable number of investigations describing new low-toxicity chemical means and reporting interesting results for decay control is available 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 it 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 overly 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. Further, 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. Additionally, other issues that hinder the commercial adoption of these alternative technologies, particularly GRAS salts, are the lack of commercial incentives for packinghouse material and service providers to promote them, as they are not often patent protected and expensive; added expenses related to energy needed to heat solution tanks and disinfect them for food safety purposes; and challenges related to periodic disposal of wastewater tank solutions, especially those with a high salt content or extremes of pH.

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. From the commercial point of view, the implementation of new coating technologies does not generally require the acquisition of new equipment or space within existing packing facilities, since many fresh produce packinghouses already employ waxing equipment. 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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