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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 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.

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

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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 °C. 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 °C, 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).