

Non-polluting chemical approaches to control citrus postharvest diseases

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Introduction

Commercial cultivars of *Citrus* spp. (Rutaceae), including oranges, mandarins or tangerines, grapefruits, lemons, and limes are the most widely produced fruits in the world. China, Brazil, and the United States (USA) lead the global production, which is mainly devoted to juice extraction, but Spain is the leading country for exports of fresh entire fruit for direct consumption. Among postharvest losses of fresh fruit, those of pathological origin are especially important because whole export shipments are often rejected by wholesale buyers when they find rotten fruits, even at low proportions. In these cases, the producer is also charged for the transport and handling costs.¹ Postharvest diseases of citrus fruits are typically caused by filamentous fungi and, according to the origin of the infections, these fungal pathogens can be classified as wound or latent pathogens. Wound pathogens infect the fruit through peel injuries inflicted in the field, during harvest, transportation, postharvest handling in the packinghouse, or commercialization. They affect citrus worldwide and are the most economically important in citrus production areas with a Mediterranean-type climate, such as Spain or California. Major species are *Penicillium digitatum* (Pers.:Fr.) Sacc and *Penicillium italicum* Wehmer, which cause citrus green and blue molds, respectively.² Latent pathogens infect flowers or young fruit in the grove, but only develop after harvest. They are particularly important in production areas with abundant summer rainfall, such as Brazil or Florida, and cause postharvest diseases such as stem-end rots, anthracnose, brown rot, black rot, and gray mold.

The management of postharvest diseases in citrus packinghouse has been for many years and still today primarily based on the application of conventional chemical fungicides, such as imazalil (IMZ), sodium ortho-phenyl phenate (SOPP), thiabendazole (TBZ), or other active ingredients and mixtures, all of them with proven efficacy against green and blue molds. However, the continuous use of these chemicals has arisen important concerns about environmental contamination and human health risks associated with fungicide residues. Updated regulations in many production areas are increasingly restricting the use of these synthetic chemicals and important export markets worldwide are demanding fruit with zero residue levels or levels lower than those established by legislative regulations. Furthermore, the widespread and repeated use of these compounds has led to the proliferation of pathogenic resistant biotypes in commercial citrus packinghouses. Therefore, there is a clear need to find and implement disease control methods alternative to conventional fungicides. In this context, research should focus on evaluating novel non-polluting alternative antifungal treatments and defining integrated disease management (IDM) programs to accomplish satisfactory decay control. Such programs are based on comprehensive knowledge of pathogen biology and epidemiology and consideration of all preharvest, harvest, and postharvest factors that may influence final disease incidence.³ According to their nature,

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alternative decay control methods can be physical, chemical, or biological. Significant advances have been accomplished in the last few years in the development and evaluation of novel non-polluting or low-toxicity chemical treatments.

Alternative chemical treatments

Low-toxicity chemicals alternative to conventional fungicides for citrus postharvest disease control should be compounds with known and minimal toxicological effects on mammals and impact on the environment. They should be, therefore, affirmed as food additives or generally regarded as safe (GRAS) substances by national or transnational legislations. They can be of synthetic origin, like inorganic or organic salts or composite edible coatings formulated with antifungal ingredients, or of natural origin, like plant extracts, essential oils, antifungal peptides, or natural antifungal edible coatings such as chitosan. Due to their general low toxicity, they are often evaluated in combination with other postharvest treatments of the same or different nature as part of 'multiple hurdle' control strategies.

GRAS salts

Sodium carbonate, sodium bicarbonate, and potassium sorbate (PS) are currently the most common inorganic and organic salts, classified as GRAS compounds, used for postharvest decay control in citrus packinghouses worldwide. They are typically used as short dip treatments in solutions at 2-3%, which significantly reduce the incidence of green and blue molds without causing rind phytotoxicities. These treatments are synergistic with heat and are often used as part of hurdle strategies. They have a relative low cost and could be of use without restrictions for many applications including organic agriculture. Because of their low toxicity, their effectiveness is clearly influenced by the host genotype and condition.^{2,4} Other food preservatives with proven activity against citrus postharvest diseases include sodium benzoate (SB), sodium paraben salts, and potassium silicate.⁵

Natural compounds

Plant extracts and particularly essential oils are the natural compounds that have been more frequently evaluated as alternative chemical means for citrus postharvest decay control. Active ingredients

are generally a combination of volatile secondary metabolites with direct activity against fungal pathogens. For instance, compounds such as acetaldehyde, benzaldehyde, ethanol, ethyl formate, hexanal, thymol, eugenol, jasmonates, glucosinolates, isothiocyanates, citral, limonene, and a variety of phenolic compounds like flavanones, polymethoxylated flavones, or coumarins have been found in oils or extracts of plant origin with antifungal activity. Among others, plants from which these compounds have been obtained include thyme, clove, oregano, mint, cinnamon, garlic, pomegranate, *Acacia* spp., *Aloe* spp. (gels used as fruit coatings), and also citrus fruits.^{3,6} In general, these compounds are considerably more effective in tests *in vitro* than *in vivo*, and other practical limitations associated with their use are the risks of phytotoxicity and induction of strong odors or flavors to treated fruit.

Some antimicrobial peptides or small proteins naturally produced by plants or animals as a defense mechanism (e.g. iturins and fengycins) have also shown activity against postharvest pathogens.⁷ Important limitations, however, are poor bioavailability, nonspecific toxicity, and low stability.

Antifungal edible coatings

Fresh citrus fruits are generally coated in packing lines with wax-based compounds, often amended with conventional fungicides such as IMZ or TBZ, to reduce weight loss, improve appearance, and control postharvest diseases. Antifungal edible coatings that are being evaluated to replace these commercial waxes include chitosan- or *Aloe vera*-based coatings, which present inherent antimicrobial activity, and biopolymer-based coatings formulated with food-grade antifungal ingredients.

Chitosan is a natural and biodegradable biopolymer obtained by deacetylation from the chitin present in the exo-skeleton of crustaceans. It shows direct antimicrobial properties against a wide range of microorganisms, including *P. digitatum* and *P. italicum*. In different research works, chitosan coatings have significantly reduced citrus molds and both direct effects on the pathogen and indirect effects on the host have been reported.^{8,9} Furthermore, chitosan also works well in combination with other control means such as biological control antagonists, GRAS salts, plant extracts, and essential oils. Some of these compounds have been incorporated into chitosan matrixes.¹⁰ In other cases, edible bilayer coatings comprised of chitosan and other polymers like carboxymethyl cellulose (CMC) have been developed.¹¹

Substantial research has been devoted in recent years to the development of novel synthetic food-grade composite coatings with antifungal properties. They are comprised of a mixture of hydrocolloids (good gas barrier characteristics) and lipids (good barrier to moisture) that form a matrix to which additional ingredients including antifungal compounds of different nature are added. The most common of these composite coatings for citrus fruits contain hydroxipropylmethyl cellulose (HPMC) or CMC as hydrocolloids, beeswax as lipid, and GRAS salts (PS, SB, paraben salts,...) or essential oils as antifungal ingredients.^{12,13} In general, these edible coatings are indicated for prolonged cold storage of citrus fruits since they satisfactorily maintain quality and reduce weight loss and postharvest decay. The incorporation of essential oils into coatings can

considerably reduce the problems associated with their application as stand-alone treatments, viz. induction of undesirable odors or flavors, phytotoxicity risks, and lack of efficacy in *in vivo* trials.

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Conflict of interest

The author declares no conflict of interest.

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