Chapter 8

Organic and Inorganic Salts as Postharvest Alternative Control Means of Citrus

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Abstract

Several postharvest disease control means alternative to conventional chemical fungicides, such as organic and inorganic salts, will be highlighted in the proposed chapter. In particular, it will comprehensively cover different aspects of the use of salts against postharvest *Penicillium* decay of citrus. It will be an essential resource for the graduate and postgraduate students, researchers, professionals, supply chain players, citrus industries, and retailers. Organic and inorganic salts have a broad spectrum of activity against a wide range of fungi. In addition, they are easy to apply, inexpensive, safe for humans and the environment, and suitable for commercial postharvest handling practices. Different application strategies of salts, before and after harvest, and combined application (with wax, natural compounds, and fungicides, etc.) will be also discussed. The present chapter attempts to highlight how the use of organic and inorganic salts as alternative postharvest disease management technologies has developed from the laboratory to the market.

Keywords: citrus, salts, postharvest pathology, GRAS

1. Introduction

Citrus world production has reached 71 M tons in 2013 (http://faostat3.fao.org/), and the Egyptian production reached 2.9 M tons, representing 30% of the total Egyptian fruit production. Italy ranked at ninth place in the world producers chart with 1.7 M tons. Citrus fruit suffers from postharvest diseases caused by several fungal pathogens. The major postharvest diseases can be divided into two classes based on their initial infections. Rots from field infection include *Alternaria* rot, *Phytophthora* rot, *Phomopsis* and *Diplodia* stem-end rot, and Anthracnose rot. Rots from postharvest infection include *Penicillium* rots, *Aspergillus* rots, Rhizopus rot, Sour rot, and Fusarium rot [1].

Green, blue, and whisker moulds caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., *P. italicum* Wehmer, and *P. ulaiense* (Hsieh, Su & Tzean), respectively, are the most important and destructive postharvest diseases of citrus fruit, especially if grown under Mediterranean climate conditions. The control of postharvest diseases is generally exerted by chemicals. However, many fungal pathogens have developed resistance to the active ingredients of a wide range of fungicides. In addition, the problems associated with their use (i.e., waste disposal), as well as the public increasing awareness about health and environmental risks, have promoted the search for new and safer alternatives. Replacement of synthetic fungicides by salts, which are nontoxic for consumers and for the environment, is gaining considerable attention.

During last two decades, because of the consistent increase in the area under citrus cultivation, the production has grown dramatically. However, the interest in protecting the fruit against postharvest losses caused by the pathogens has not gained the same spur in the implementation of the available technologies. Despite international organizations responsible for monitoring food resources have been recognized that the most economically feasible and expedient means to increase food supply are to reduce postharvest losses [2].

The surface of fruits or vegetables is covered by fungal and bacterial propagules; however, most of them do not cause decay, even when conditions suitable for penetration and development are present because of natural resistance. However, natural resistance to disease of fruits generally declines after harvest, due to ripening and senescence, leading to decay and physiological impairment. The preharvest factors (soil conditions, moisture, temperature, relative humidity, and nutrient availability, etc.) that affect the postharvest life of a fresh produce were discussed [3].

2. Penicillium decay

The genus *Penicillium* includes 150 species, but only few species are economically important plant pathogens [4]. In Mediterranean climate areas, green and blue moulds caused by *P. digitatum* and *P. italicum*, respectively, are the most important postharvest diseases of citrus fruit. Both fungi are necrotrophs that feed on the dead matter and require wounds to enter the fruit [5]. Blue mould is generally of lesser overall importance, but may become a major problem under prolonged storage conditions, which suppress development of green mould [6]. At room temperature, green mould invades fruit much more rapidly than blue mould and predominates in mixed infections [7]. Currently, another species of *Penicillium*, *P. ulaiense* causing whisker mould, has being considered important on citrus, especially in packinghouses. It is a pathogenic fungus described as a member of the series Italica [8], together with *P. italicum*. In the past, the fungus was mistaken for *P. italicum* and its unique features dismissed as variations due to particular environmental conditions [9]. Recently, this fungus is becoming a serious concern for citrus packinghouses in several countries, because of its resistance to the commonly used preservatives. Rots are differentiated from those of *P. italicum* by the presence of coremia on the fruit [10]. Initially isolated from decaying oranges in Taiwan [11], *P. ulaiense* has been described on citrus fruit in the United States [9, 10], Australia [12], Argentina [13], and recently in Egypt [14] and Tunisia [15]. Besides the taxonomic and phytopathological aspects [8, 10], there is little or no information about this organism [16].

The optimal growth conditions for *Penicillium* involve a warm and moist environment. Although infection may take place in the field, blue and green moulds are essentially postharvest pathogens. The fungus can spread during storage, since the airborne spores are long-lived and may easily survive from season to season on contaminated bins. Inoculation can also occur during fruit handling, for example, in water contaminated with the pathogen spores in packinghouses [17]. A single decayed fruit may contain enough spores to contaminate water of the entire packing line [18]. The pathogen can penetrate the host tissues also through openings in the peel, and can spread from one fruit to another by simple contact (nesting). When the host is first infected, a stain appears on the peel; this rapidly forms a slight indentation, after which the fruit decomposes partially or totally within few days.

3. Disease management of citrus fruits

The best and most effective management strategies to control citrus fruit diseases would be to avoid or minimize the predisposing factors responsible for origin and development of the diseases through an integrated approach. These management strategies include improved cultural practices, regular monitoring of the disease appearance and weather conditions, improved postharvest handling, control agents, timely use of effective fungicides, transport, and storage conditions.

3.1. Accurate cultural practices

Efficient removal of infected fruit and minimizing fruit injury are effective ways to control blue and green moulds. Disinfectants such as chlorine, quaternary ammonium compounds, formaldehyde, and alcohol are useful for preventing inoculum buildup. Spore populations are kept low in packing areas by removing infected fruit promptly, using exhaust fans, and keeping dump areas well away from packing facilities [19]. Harvest following rain is discouraged because wet fruits are more prone to injury. However, sanitary practices should be applied to prevent sporulation on diseased fruit and the accumulation of spores on equipment surfaces as well in the atmosphere of packing and storage facilities. Efficient packinghouses systematically segregate spoiled fruit in storage, distribution or repack, effectively reducing the disease [20, 21]. Moreover, effective sanitation practices during pre and postharvest handling, avoiding possible inoculum sources and preventing contamination, can significantly reduce the incidence of decay.

3.2. Chemical control means

Control of green and blue moulds varies among different countries' legislation but is mainly based on the use of fungicide treatments, principally thiabendazole and imazalil, sprayed alternately or simultaneously, on fruit during waxing operation in the packinghouse. The control of postharvest fungal pathogens on citrus depends on the prompt application of a suitable fungicide at its recommended concentration. If the fungicide is not applied soon after the rind injury, which usually occurs during harvesting and subsequent handling, infection establishes and the organism escapes the protective activity of the compound being applied. The time allowed between the beginning of an infection and the application of a fungicide to completely inhibit or control infection depends on the growth rate of the germinating organism, which in turn depends on the fruit temperature [22]. Fungicidal dips and sprays, with thiabendazole, imazalil, thiophanate-methyl, sorbic acid, guazatine or sodium o-phenylphenate (SOPP) are used to control the disease [23]. Several years after the introduction of fungicides as thiabendazole, imazalil, and SOPP, resistant biotypes of *P. digitatum* were reported in packinghouses in many citrus production areas [24] and terminal markets [25]. These fungicides are used in a manner that is highly conductive to the selection and proliferation of resistant strains of *P. digitatum* and *P. italicum*. O-phenylphenol and thiabendazole have been used routinely on citrus fruits over the past three decades, resulting in a serious problem of resistance by the late 1970s [26]. It was reported that imazalil, successfully used in Mediterranean citrus production areas for several years, could be an effective treatment for control of thiabendazole-resistant isolates of *P. digitatum* and *P. italicum* [27]. It was investigated that all *P. digitatum* and *P. italicum* isolates collected in a citrus orchard were sensitive *in vitro* to imazalil and thiabendazole [28]. This result was consistent with earlier studies [29–31] suggesting that naturally occurring resistant isolates of *P. digitatum* and *P. italicum* are rare or absent in citrus orchards, especially in those orchards without a prior history of fungicide usage. Nevertheless, isolates of *P. digitatum* resistant to thiabendazole, imazalil, or both fungicides were detected in all packinghouses sampled. Pyrimethanil (PYR) has been registered in USA against citrus green and blue moulds. A 90% reduction of green mould by PYR applied at ≥500 mg/l by dipping or drenching the fruit, and a 65% reduction applying PYR at 1000 or 2000 mg/l in wax over rotating brushes were observed. Indeed, PYR in aqueous solutions controlled sporulation better than when applied in wax, but it was less effective than imazalil, although an imazalil-resistant *P. digitatum* isolate was controlled by PYR. The sodium bicarbonate addition proved to improve PYR performance [32].

3.3. Nonchemical control means

Although synthetic fungicides are the primary means for controlling postharvest diseases of fruits and vegetables, the growing concern for the human and environmental health [33], the cost of developing new pesticides to overcome pathogen resistances [34, 35], and the lack of continued approval of some of the most effective fungicides motivated the search for alternative approaches [36]. Several alternative means have been proposed to control postharvest diseases of fruits and vegetables: biocontrol agents [37], natural substances [38], physical treatments [39], and organic or inorganic salts [40–42]. Although all these approaches proved to be effective on a large number of hosts, they do not always offer a control level comparable to that provided by synthetic fungicides. However, an economically sufficient control extent can be obtained by the use of two or more alternative means in an integrated approach [37].

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3.3.1. Generally recognized as safe (GRAS) substances

"Generally recognized as safe (GRAS)" is a category of the American Food and Drug Administration (FDA), stating that a chemical is considered safe to humans and animals, and thus considered food-grade. As such, carbonic acid salts are GRAS additives allowed with no restrictions for many applications (including food industry) in Europe and North America. The antimicrobial activity of these chemicals has been described *in vitro* [43] and in a wide range of substrates as well. Sodium bicarbonate ($NaHCO_3$) has been used as a disinfectant for citrus fruit since 1920 [44–49]. Treatments with sodium carbonate (Na_2CO_3) and $NaHCO_3$ that reduced the incidence of postharvest decay on lemons proved to be as effective as higher concentrations of calcium chloride ($CaCl_2$) in preserving tissue firmness during storage [50–52]. Inversely, Ca salts at high concentrations (187.5 mM) caused symptoms of phytotoxicity on the fruit surface, in terms of skin discoloration and superficial pitting, leading to further chemical changes and reduced tissue firmness [53]. Concerning strawberries, $CaCl_2$ dips in combination with heat treatment or storage in modified atmosphere and refrigeration proved to increase calcium content, fruit firmness, and delay postharvest decay [54]. Organic calcium salts are an alternative calcium source and calcium lactate has been described in the literature as a firming agent for several fruit. According to Lawles, the bitter and salty tastes associated with calcium chloride are largely suppressed when calcium is combined with larger organic ions such as lactate, gluconate, or glycerophosphate [55].

In Egypt, a complete inhibition was observed in the linear growth of *G. candidum*, *P. digitatum*, and *P. italicum* when exposed to benzoic, citric, and sorbic organic acids at concentrations of 4% and 2% of either sodium benzoate or potassium sorbate, respectively [56]. The efficacy of potassium sorbate and ammonium bicarbonate as possible alternatives for controlling soil-borne pathogens *Fusarium oxysporum* f. sp. *melonis*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* was evaluated [57]. Authors summarized that potassium sorbate had higher toxicity to all fungi compared to ammonium bicarbonate in soil tests. Both ammonium bicarbonate and potassium sorbate increased the pH of soil. The rate of pH increase was higher in ammonium bicarbonate.

More than 20 food additives and GRAS compounds were tested to control major postharvest diseases of stone fruit by Ref. [58]. Overall, the best compounds were 200 mM potassium sorbate, 200 mM sodium benzoate, 200 mM sodium sorbate, 100 mM 2-deoxy-D-glucose, 400 mM sodium carbonate, and 250 mM potassium carbonate. Moreover, potassium sorbate was successfully tested in mixture with commercial fungicides against the major postharvest pathogens of citrus fruit, in particular, combined low concentrations of potassium sorbate with imazalil, thiabendazole, pyrimethanil, and fludioxonil [59]. Potassium sorbate was not only compatible with these fungicides, but also enhanced their effect against *P. digitatum* and *G. citri-aurantii*. In other studies [40, 60–62], potassium sorbate solutions applied at room temperature or moderately heated for relatively long immersion times (2–3 min) resulted effective to control green mould [59].

3.4. Integrated control options and strategies

Alternative control means alone are often less effective compared with commercial fungicides or provide inconsistent control. Therefore, to achieve a similar level of efficacy provided by conventional fungicides, an integration of commercial chemicals at low doses [63], hot water [64], chloride salts [56, 65], carbonate salts [40], natural plant extracts [66], and other physical treatments such as curing and heat treatments [67], is recommended [68–71].

3.4.1. Citrus fruit wax combined with salts

Generally, citrus fruits are waxed in order to improve their shine [72] and reduce water loss during postharvest storage. Previous studies showed that, the application of shellac-based waxes reduced internal O_2 levels, and increased internal CO_2 and ethanol levels [73]. According to Waks, waxes minimize stem-end rind breakdown and other collapses of rind tissue, and can protect the fruit from the entrance of pathogens. The effect of waxing on the incidence of postharvest rots apparently is not unique to citrus fruits [74]. For example, it is reported for Starking apples attacked by *Gloeosporium* sp., the cause of apple bitter rot [75]. Coating (mainly with wax, shellac, and sucrose ester, etc.) is not recommended for long-term storage to prevent off-flavor [76].

Waxes may also serve as carriers of fungicides or growth regulators, because these chemicals are less effective when applied in waxes than when applied in water; therefore, higher concentrations are generally used in waxes than in water [77]. According to Taverne, when combined in wax and applied at label rate (150 μL/100 g), imazalil reduced the decay levels. In particular, when fruits were treated with wax containing ≥3000 ppm of imazalil, sporulation was reduced, since residues of 2–3 ppm were achieved. Doubling the wax volume did not significantly improve the sporulation control. Combinations of imazalil applied in water followed by imazalil in wax might be required to ensure high decay and sporulation control [78].

It evaluated the effect of organic acids (ascorbic, benzoic, citric, and sorbic) as well as organic salts (potassium sorbate and sodium benzoate) at different concentrations (0, 0.5, 1.0, 2.0, and 4.0%) on the growth of *Geotrichum candidum*, *P. digitatum*, and *P. italicum in vitro* and *in vivo*. A complete inhibition was observed in the linear growth of all tested fungi when exposed to all organic acids concentrations and to 4% and 2% of either sodium benzoate or potassium sorbate salts, respectively. The various tested organic acids and salts showed different extent of protective or therapeutic effect on the coated lemon fruit against mould infection, whatever the time of their artificial inoculation. All treated citrus fruit showed reduced rate of sour rot, green and blue moulds when compared with untreated fruits. A complete inhibition of mould incidence was obtained in coated lemon fruits with 4% of sodium benzoate and potassium sorbate in water or wax mixtures 24 h before inoculation. In addition, high reduction in mould incidence was observed in lemon fruits coated with the same salt concentration 48 h after inoculation under the same conditions [56].

In general, the most practical combination of solution temperature, chemical concentration, and treatment duration for optimal decay control must be determined for each chemical and each host-pathogen systems [68]. Heat and the integration of certain physical, chemical, or biological treatments have been evaluated for postharvest decay control of peaches and nectarines [79, 80]. According to our knowledge, a limited research has been conducted to elucidate the ability of a mixed application of wax and food additives on the development of postharvest diseases of citrus during storage [41].

3.4.2. Electrolyzed water in combination with salts

Sanitizing is considered a fundamental component of processing the fresh fruit and vegetables. Typically, chlorine compounds and fungicides are the main active elements in sanitation and postharvest processing of fresh fruits. The corrosive effect of chlorine and toxicity of fungicide residue are considered a serious issue in processing fresh products [81, 82]. Development of alternative methods for sanitizing fresh products and controlling postharvest diseases is derived by economic and consumer demand motivations [83].

Electrolyzed water (EW) was introduced as decontaminating agent [84], and its application was approved in Japan and USA as a food additive and sanitizing agent [85, 86]. EW has shown its potential to inactivate pathogenic microorganisms such as bacteria [87–89] and fungi [17, 90–93]. One of the main advantages of EW is the less adverse effect on environment and human health because no hazardous materials are used during its production [85].

The electrolysis of diluted solution of salts (e.g., NaCl, KCl, and $MgCl_2$) leads to dissociation of salt ions, and formation of anion and cations at anode and cathode, negative and positive electrodes, respectively [94]. The physical properties and chemical composition of EW vary depending on the concentration of salt solution (e.g., NaCl), electrical current, length of electrolysis, and water flow rate [95]. Most previous studies assessed electrolyzing parameters (flow rate, current intensity, and time for electrolyzing) effect on free chlorine, electric conductivity, and pH of the resulted EW [96–98], while very few studied the effect of electrolyzing parameters on the ability of electrolyzed water to deactivate pathogen unit [17]. Although salt solutions used in electrolysis play a major role in the effectiveness of EW, few salts were studied for their effect on EW efficiency: sodium chloride [93, 96–100], potassium chloride [101], and sodium bicarbonate [102].

Anions of Cl^- are targeting polypeptides and carbohydrates in the cell wall, in addition to destruction of nucleic acids—DNA and RNA—which impairs the process of DNA replication and gene expression, therefore, halts the cell division and stops the essential biological function leading to cell death. Oxidation Reduction Potential (ORP) has shown moderate biocide effect following free chlorine level. ORP indicates the tendency of ions to accept/donate electrons (reduction/oxidation), and was suggested as alternative stronger sanitizing factor compared to free chlorine [91, 103, 104]. It was found that in case of long exposure time, decrease of ORP to −300 mV reduced coliform bacteria population to 10 fold, and at ORP −400 mV, coliform bacteria population decreased 100 fold, and decreasing ORP to below −600 mV will completely inactivate all coliform bacteria [105]. Thus, it was possible to improve the biocide activity by manipulating the ORP regardless the anion present. Using salts with different anion radical than Cl^-, it is possible to still achieve the same level of disinfestations or even improve the biocide activity targeting multiple sites in the pathogen biological system [105].

The values of pH represent the level of hydrogen ion activity governing the solution acidity/alkalinity. The solution acidity/alkalinity has a biocide effect against pathogen cells, and although it came third to free chlorine and ORP, it still shows significant effect on the pathogen count. The role of pH is believed to make the cells more sensitive to active chlorine by erupting their outer membrane and facilitating the entry of HOCl [106]. Previous papers have studied the effect of electrolyzing parameters on the electrolyzed water characteristics [17, 95]. Recently, acidic electrolyzed water has shown higher biocidal activity toward *P. digitatum*, *P. italicum*, and *P. ulaiense* causing green, blue, and whisker moulds, respectively. When compared to the effect of alkaline electrolysis water [107], the author suggested that using amended salt solutions would improve the biocidal effect of electrolyzing. Further studies have suggested the higher biocide effect of acidic water and also suggested alkaline water to be used in cleaning and degreasing before application of acidic electrolyzed water [108–112].

3.5. Preharvest treatment for controlling postharvest citrus decay

Citrus requires about 5–9 months for its maturity on trees, and during this long maturity period, the fruit remains exposed to the attack of preharvest fungal pathogens such as *Colletotrichum gloeosporioide*s, *C. acutatum*, *Botryodiplodia theobroma*e, *Alternaria citri*, and *Phomopsis citri*, etc. The incipient infection of preharvest pathogens subsequently manifests in the form of postharvest diseases, besides the attack of the main postharvest pathogens such as *P. digitatum*, *P. italicum*, and *G. candidum*, etc., during postharvest handling, transport, storage, and marketing. A number of nonconventional alternatives have been trailed as standalone treatments. It was showed that, potassium sorbate was effective when used on oranges, grapefruits, mandarins, and tangerines, but later research found that the effectiveness was influenced by citrus species and cultivars [60]. Potassium sorbate efficacy is variable when used as commercial treatment for citrus fruits, limiting its use as a standalone treatment [113].

In this perspective, the optimization of some promising alternative methods, like the use of salts, is a great interest. Since injuries sustained by citrus fruit during harvest, strongly favorite wound pathogens such as *P. italicum* and *P. digitatum*, the reduced efficacy of alternative control means could be partially ascribed to the lack of curative effect against already established infections. Based on this consideration, a near-harvest treatment could be an appropriate strategy to prevent the colonization by pathogens during harvesting and postharvest handling of fruits [42, 114].

The effectiveness of different salt solutions (2%, w/v), sodium carbonate and bicarbonate, potassium carbonate and bicarbonate, and calcium chloride, in controlling postharvest *Penicillium* rot of "Hernandina" clementine were reported [115]. Preharvest sprays and the combination of pre and postharvest application of salts were in average more effective, in suppressing *Penicillium* rot, than the individual postharvest dipping [42]. A detailed study of the ability of sodium carbonate and bicarbonate to induce resistance in oranges has been performed and published [116].

4. Conclusion

The book chapter aims at presenting salts as a sustainable approach for controlling postharvest diseases of citrus fruit to researchers and industries, graduate, and postgraduate students. The use of organic and inorganic salts was reviewed since the pioneer studies in the early 1920s of the last century until the most recently published works. Several salts confirmed their efficacy as affordable, safe to consumers and operators, and already used in the food industry. More studies concerning their mode of action might contribute to expand their application in the field and/or packinghouses.

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