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Chapter

Alternative Eco-Friendly Methods in the Control of Post-Harvest Decay of Tropical and Subtropical Fruits

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Abstract

The effectiveness on several fruits by the application of alternative methods against fungi is summarized in the present chapter. Several investigations have reported the efficacy of these technologies for controlling fungal infections. Currently, high post-harvest loses have been reported due to several factors such as inefficient management, lack of training for farmers, and problems with appropriate conditions for storage of fruits and vegetables. Even now, in many countries, post-harvest disease control is led by the application of chemical fungicides. However, in this time, awareness about fungi resistance, environmental, and health issues has led to the research of eco-friendly and effective alternatives for disease management. The pathogen establishment on fruits can be affected by the application of GRAS compounds like chitosan, essential oils, salts, among others; besides, their efficacy can be enhanced by their combination with other technologies like ultrasound. Thus, the applications of these alternatives are suitable approaches for post-harvest management of fruits.

Keywords: alternative systems, antifungal activity, postharvest fungi, tropical and subtropical fruits

1. Introduction

The consumption of fresh fruits and vegetables is essential for a healthy diet [1]. However, their production can be affected by microbial pathogens (mostly fungus)

during the production chain [2]. In order to reduce the presence of pathogens, several post-harvest technologies have been applied [2]. One of them is the application of chemical fungicides; however, this practice is not accepted due to environmental and health issues [3]. Other alternatives are the use of eco-friendly substances such as generally recognized as safe (GRAS) compounds and emergent technologies like ultrasound and fogging. Currently, consumers demand fresh products free of chemical residues; therefore, it is necessary to develop technologies eco-friendly, effective to protect against pathogens infection, and that these technologies can maintain the fruit quality. Alternative systems such as edible coatings, essential oils, salts, natural compounds (plant extracts), among others to chemical use are suitable approaches for post-harvest disease management. These alternatives can be applied in combination with other control systems like emerging technologies (ultrasound) in order to improve their efficacy. The aim of this chapter is to make a compilation of several studies conducted on fruits for controlling important pathogens in several crops.

2. Fruit industry: importance in the world

Fruits and vegetables are essential sources for the micronutrients needed for healthier diets [4]. The potential of vegetables is to generate positive economic and nutritional impacts. The estimated farm gate value of annual global fruit and vegetable production, at nearly $1 trillion per year, exceeds the farm gate value of all food grains combined (US$ 837 billion). On the other hand, it is likely that the production of fruit and vegetable crops will not increase as rapidly as would be expected. Environmental changes can affect many different aspects of agricultural production. With greater climatic variability, temperature patterns and precipitation are some of the problems faced by fruit producers [5]. Technological advances have focused their efforts on the development of new varieties, crop management techniques, and innovations in postharvest handling and processing. Even in high-income countries such as the US, there is evidence that public funding for research in the agricultural area is less than expected, given its economic value and its contribution to human health [6].

3. Post-harvest losses: tropical and subtropical fruit

It is reported that about one-third of the production of food intended for human consumption is lost or wasted worldwide, which is roughly equivalent to 1.3 billion tons per year. This means that huge amounts of resources directed to food production are used in vain, and that greenhouse gas emissions caused by food production that is lost or wasted are also unnecessary [7]. A very significant part of the food that deteriorates or that is lost at post-harvest stage are fruits and vegetables. These losses occur throughout the management system of fruits during the harvest, transfer to the packinghouse, in the packing, during the storage, transportation and distribution to marketing centers. The causes of the losses in post-harvest are due to economic limitations, the lack of post-harvest technology as well as the lack of trained personnel about the knowledge in technology, management, physiology, and post-harvest pathology of horticultural products. This problem occurs mainly in developing countries, reaching up to 30 or 40% of post-harvest losses [8].

The fruits during their growth in the field are exposed to the attack of pathogenic fungi; they can be established as a latent infection in the fruit; and when the fruit begins its maturation process, the fungi can be activated and continue with their development, leading the deterioration of fruit. To control these pathogens, synthetic fungicides have been applied in a traditional manner; however, due to pollution and the environment issues, damage to the health of people and in general to living beings, as well as generating resistant strains of fungi, it is necessary to find alternatives to pathogen control [9, 10]. Currently, the research of alternative systems to the use of fungicides to control the losses caused by fungi, applying products of biological origin as well as organic and inorganic salts, among others, in order to control fungi infections, without contaminating the environment and without harming living beings has increased [11, 12]. Reducing the losses of post-harvest could solve the problem of hunger in many countries of the world, where not only it is producing more food but rather it is to conserve the food that is currently produced.

4. Chemical methods: applications for post-harvest disease management

Most of the post-harvest losses are attributed to the attack of a large amount of fungus in tropical and subtropical fruits. Chemical control of post-harvest diseases is widely used to maintain fruit quality [13]. There are a wide variety of fungicides for chemical control, and the vast majority is destined or directed to the pre-harvest applications, leaving aside the use in post-harvest stage [14, 15]. To make efficient the use of chemical fungicides, it is necessary to know both the pathogen and the fungicide. From the pathogen, it is necessary to know the genus and the species as well as their concentration found at pre- and post-harvest stages. On the part of the fungicide, it is necessary to know the mode and site of action, as well as the maximum residual limit (MRL) (**Table 1**) permitted on fruits and specific regulations where the fruit will be exported [16]. In **Table 1**, some fungicides used for post-harvest disease of tropical and subtropical fruit diseases are listed. The site and mode of action are summarized in the table, as well as the MRL that are allowed in the US [17] and the EU [18]. An important consequence for the inadequate use and irrational applications of chemical treatments is microbial resistance, and in this sense, it is recommended to alternate formulations to avoid this problem. Besides, post-harvest chemical control should be regionalized to the specific conditions and environment of each crop [19]. It is important to mention that agrochemical companies suggest the doses and formulations to use in a specific crop. At this point, public research centers have an important contribution, not only to verify the efficiency of fungicides but also to establish strategies for the efficient use of post-harvest chemical control [15]. Considering the new consumer tendencies, about secure products free of chemical residues, it is necessary to consider the rational use of chemical fungicides without exceeding the MRL [20–22].

Therefore, the worldwide trend for both consumers and researchers is the reduction of the use of chemical fungicides and the research for biological, organic, and environmentally friendly alternatives. All this is under certification systems to guarantee the implementation, improvement, integration, and harmonization of all mechanisms to ensure a production of healthy and good quality fruit, with high traceability (GLOBALG.AP) [23, 24].

Common name	MRL (ppm)						Chemical group	Mode action	Target site
	EU [17]			US [16]					
	Avocado	**Mango**	**Papaya**	**Avocado**	**Mango**	**Papaya**			
Cyprodinil	1.2	1.2	1.2	1	1	1	Anilino-pyrimidines	Amino acids and protein synthesis	Methionine biosynthesis (proposed)
Carbendazim	—	—	—	3	2	3	Benzimidazoles	Cytoskeleton and motor proteins	ß-tubulin assembly in mitosis
Thiabendazole	10	10	5	3	3	10			
Pyraclostrobin	0.6	0.6	0.6	—	0.05	0.2	Methoxy-carbamates	Respiration	Complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site
Trifloxystrobin	—	0.7	0.7	—	0.7	—	Oximino-acetates		
Fludioxonil	5	5	5	5	5	5	Phenylpyrroles	Signal transduction	MAP/Histidine-Kinase in osmotic signal transduction
Iprodione	—	—	—	10	10	10	Dicarboximides		
Prochloraz	—	—	—	5	2	1	Imidazoles	Sterol biosynthesis in membranes	C14-demethylase in sterol biosynthesis
Difenoconazole	—	0.1	0.6	0.6	0.07	0.2	Triazoles		

Table 1.
Common name, chemical group, mode and target site as well as its MRL in some fruits for consumption [23].

5. Alternative methods in the control of post-harvest decay of tropical and subtropical fruit

5.1 Chitosan

The excessive use of agrochemicals in tropical and in subtropical fruit has leaded the search for new natural products, eco-friendly, and nontoxic to humans. In several investigations, chitosan has proved their efficacy for controlling several post-harvest diseases. Several mechanisms of action have been proposed for chitosan:

a. Pathogens: the interaction of the biopolymer with the microorganism causes changes on cell permeability affecting biochemical processes like homeostasis, fungal respiration as well as nutrient uptake and the synthesis of proteins causing severe damage on fungal cells [12].

b. Plants: induction of defense systems, by the production of important enzymes (phenylalanine ammonium lyase, polyphenol oxidase, among others) and plant immunity, favoring the adaptation of plants to biotic and abiotic stresses [25].

c. Fruits: the capability of chitosan to form mechanical barrier (coating) on fruits offers several advantages of coated fruits like a reduction on respiration rate, avoid water losses maintaining fruit firmness, maintenance of color, among others. Thus the shelf-life of fruits can be extended [36].

The induction of defense systems has been reported by the application of chitosan at post-harvest stage, preventing the development and dispersion of important pathogens such as *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Rhizopus stolonifer*, and *Fusarium oxysporum* [26–28]. Enzymatic activity is also affected by the curative application of chitosan, and it increases the activity of polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine amino-lyase (PAL) that induce the expression genes of β-1,3-glucanase and chitinase, involved in the defense against pathogens [29, 30]. The physiological mechanisms of the fruit are positively affected by the application of chitosan at post-harvest management under biotic and abiotic stress, that is why the post-harvest shelf-life and quality (firmness, appearance, color) of fruit can be maintained during the storage time, besides the respiration rate and ethylene production of fruits decrease [31]. Some studies reported an enhanced content of total soluble solids, ascorbic acid, the nutritional value, and acceptability [30, 32, 33]. Chitosan is compatible with other substances like organic salts, gums, or essential oils, and this alternative can improve their efficacy against pathogens due to a synergistic effect [34, 35]. Even when important information has been generated on the use of chitosan in post-harvest tropical and subtropical fruits, it is still necessary to generate information on the regulation (activation and suppression) of genes that participate in both systems of acquired resistance and those that control the processes physiological, enzymatic, and physicochemical factors of maturation in post-harvest.

5.2 Essential oils

The use of GRAS substances like essential oils (EOs) has increased in the last years, due to the research of alternatives to chemical treatments for disease control [36]. Several investigations have reported the efficacy of essential oils *in vitro* and *vivo tests* [37–40]. The application of EOs in fruits has advantages such as: high effectiveness against several pathogens and low toxicity (nontarget microorganism and humans) [36]. EOs can be applied on fruits directly (vapor phase) or incorporated as active

microbial agents in different matrices (films, coatings, among others) at pre-harvest or post-harvest stages. In a recent investigation, cinnamon essential oil was added into biodegradable polyester nets for controlling *A. alternata* [41]. The results are promising due to their efficacy *in vitro* tests by stopping the development of fungus on mycelial growth (72% of inhibition) and the total control of germination. The presence of essential oil did not alter the biodegradability of nets as well as their efficiency to maintain fruit quality and the disease control on infected fruits by reducing the disease incidence. Incorporation of EOs into edible films for active packaging has demonstrated high efficacy against important post-harvest pathogens. Recently, soy protein isolate was used as a carrier for limonene with good results against *P. italicum* (isolated from infected limes) [42]. The liberation and efficacy (*in vitro* tests) of the active agents were evaluated simulating the storage conditions of limes (13 and 28°C). The mycelial growth and germination process were successfully inhibited by the incorporation of limonene into the protein matrix. The results *in vitro* were confirmed in infected limes with *P. italicum* by reducing the severity and blue mold incidence with the application of soy protein isolate with limonene added [43]. In another study, films based on chitosan, oleic acid/beeswax, and lemon essential oils were tested on tomatoes for preserving their quality. According to their results, the applications of these films improve the fruit quality by reducing water losses and the maintenance of appearance of fruit [44]. In a recent study, anthracnose and stem-end rot in the green-skinned on avocado fruits was successfully controlled by the application of thyme oil in combination with a prochloraz solution; besides, this treatment improved fruit quality (firmness) during the storage time [20]. Essential oils of copaiba and eucalyptus were tested against *Alternaria alternata* and *Colletotrichum musae* under in vitro, and the results showed good efficacy at low concentrations of the treatments (0.0–1.0%) [45].

The efficacy of black caraway (*Carum carvi*) and anise (*Pimpinella anisum*) essential oils was tested against *Penicillium digitatum in vitro* as well as *in vivo* (on oranges) evaluations. The results showed that treatments were capable to control fungi development *in vivo* and *in vitro* tests; besides, the quality of oranges was preserved by the application of the treatment of 600 μL/L [46]. In a study, lemongrass oil was tested against *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer*, and *Aspergillus niger in vitro*. According to their results, fungal development, sporulation process as well as spore germination of fungi was affected in different levels (depending on the concentration of treatment) when they were exposed to the treatments by stopping their development [47].

Thus, utilization of EOs for controlling diseases can be alternatives to chemical treatments.

5.3 Plant extracts

Currently, the study of fruits and vegetables at post-harvest stage has focused on the development of alternative control for conservation with better efficiency, sustainability, and lower cost than conventional methods. In this sense, some extracts from plants have proven to be a viable alternative for the extraction of substances with antimicrobial activity with high efficiency and low toxicity [48]. In a study, aqueous and ethanolic extracts from garlic (*Allium sativum*) were evaluated on citrus fruits (*Citrus sinensis*, *Jaffa*, and *Valencia*) with good results (**Table 2**), and it is suggested that the highest antifungal activity of the extract can be produced by the presence of allicin in the soluble fractions of extracts from garlic [49]. In the same way, extracts of garlic, ginger, and celery have been shown to have different effects on the control of the incidence of *Penicillium* sp. in fruits of the species *Citrus reticulata* Blanco [50]. In a similar study, soybean extracts were evaluated as protection method on oranges infected with *P. digitatum*, and green

Fruit	Disease	Pathogen	Extract source	Disease inhibition (%)	References
Orange	Blue mold	*P. italicum*	Garlic	92	[47]
	Green mold	*P. digitatum*	Garlic	90	[47]
			Soybean	100	[49]
	Citrus rot	*Penicillium* sp.	Garlic	80.2	[48]
			Celery	5.3	
			Ginger	16.3	
Cherry	Brown rot	*M. laxa*	*O. crenata*	76	[51]
			S. minor	89	
Nectarine	Brown rot	*M. laxa*	*O. crenata*	75	[53]
			S. minor	100	

Table 2.
Disease control of plant extracts.

mold was significantly reduced (88–100%) due to the presence of β-conglycinin in the soy protein fraction [51]. In recent years, extracts of some angiosperm species have been studied as natural fungicides, such as *Orobanche crenata* and *Sanguisorba minor*, which have shown high efficiency in the control of the diseases produced by *Monilinia laxa* in stone fruits such as apricot, cherry, and nectarine [52–54]. For the case *O. crenata* extracts, the antifungal activity is attributed for the presence of the phenolic compound verbacoside, and for *S. minor* extracts, the efficacy is related to the presence of a combination of phenolic compounds like caffeic acid, quercetin, luteolin, and kaempferol [52–54].

An important approach in fruits is quality. In this sense, the application of some plant extracts can help preserve the fruit quality and improve their shelf life. In a recent investigation, the application of guava extracts (from leaves) and lemon on banana fruits (*Musa sapientum* L.) at post-harvest stage considerably improved the shelf life of the fruits (up to 8 days) compared to untreated fruits (only 4 days), having a positive effect on the conservation of the physicochemical characteristics of fruit during the storage time [55]. Utilization of plant extracts for disease management in both pre- and post-harvest stages can be another alternative to the use of chemical treatments, due to their effectiveness and eco-friendly characteristics.

5.4 Acetic acid, peracetic acid, and hydrogen peroxide

The use of sanitizing agents such as acetic acid ($C_2H_4O_2$), peracetic acid ($C_2H_4O_3$), and hydrogen peroxide (H_2O_2) on the processing and marketing industries of fruits and vegetables have been considered a useful tool to the control of different kinds of pathogens [56]. The FDA has approved the use of these compounds because their decomposition products are water, oxygen, and acetic acid, and these are not toxic compounds and are friendly with the environment [57, 58]. For these reasons, they are classified as GRAS [56]. The acetic acid, peracetic acid, and hydrogen peroxide are recognized as antimicrobial and antifungal agents, because they have high spectrum of attack on bacteria, fungi, spores, and viruses. *In vivo* studies realized on fresh and fresh-cut horticultural products confirm their antimicrobial capacity with the reduction of human pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutant*, *Salmonella Thompson*, and *Listeria monocytogenes* [59, 60]. The application of acetic acid, peracetic acid, and hydrogen peroxide also decreases the microbial pollution of aerobic mesophiles, molds, and yeast, obtaining with

these innocuous and acceptable products to the consumers [61]. In fruits like guavas, peaches, and tomatoes, the antifungal effect of these sanitizers has been confirmed with the inhibition of phytopathogens such as *Rhizopus stolonifer*, *Monilinia fructicola*, *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani*, and *Rhizoctonia solani* [62–65]. The inactivation capacity of these compounds on pathogens is based on their high oxidizing power, producing reactive oxygen species (ROS) that generate instability in biomolecules such as DNA, lipids, and proteins, which are vital for the correct cellular functioning of pathogens [56]. Usually, the application of these sanitizers at post-harvest stage in fruits and vegetables is by spraying, dipping, and fumigation. Several authors have investigated the pathogen inhibitory effect of each application system, with the finality to know which application system has better efficiency. The inhibition of *A. alternata* and *B. cinerea* in tomato fruits treated with acetic acid by immersion (50 ml/L by 3 min) and fumigation (50 μl/L by 30 min) was evaluated [64]. The results of this investigation showed the efficacy of both application systems. The growth inhibition of the pathogens tested ranged from 90 to 100% by immersion and fumigation, respectively. In strawberry fruits infected with *B. cinerea* and treated with peracetic acid, where the lower incidence of gray mold disease was obtained with the fumigation system (66%) compared to immersion method (80%) [66]. These results confirm that the capacity of inhibition of these GRAS substances against phytopathogens depends not only on the concentration used but also on the exposure time, the microorganism tested, and the application method [67]. The individual application of acetic acid, peracetic acid, and hydrogen peroxide has controlled microbial contamination in an acceptable way, but different reports have been showed that their combination with other compounds and technologies increases microbial control. Some of the technologies used include ultrasonic, organics salts, essential oils, ultraviolet light, hot water, and steam [63, 68–73]. Maintaining not only the safety but also the quality on post-harvest products is a constant challenge for the horticulture industry. The application of acetic acid, peracetic acid, and hydrogen peroxide can also improve the quality of the products, according to different in *vivo* studies carried out. In peppers, fruits treated with the solution of hydrogen peroxide and applied by dipping (15 mM by 30 min) increased their shelf life and the fruits maintained their appearance after 2 weeks stored at 20°C compared to control fruits [74]. In according to their results, fruits and vegetables such as tomatoes [75], grapes [76], nectarines [63], apples [68], and cherries [77] have been maintained the quality. Thus, the application of these sanitizers in post-harvest is a practical strategy to maintain the safe and quality of the post-harvest products.

5.5 Salts: organic and inorganic

Nowadays, there is a tendency to reduce the use of synthetic fungicides in agriculture. In this sense, organic and inorganic salts are chemical substances that are food additives generally recognized as safe (GRAS). They are widely used in the food industry due to the low toxicity and environmental impact, besides they can be combined with other control systems to control phytopathogens in post-harvest stage in different fruits and vegetables [78, 79]. It has been shown that salts such as calcium propionate completely inhibit the mycelial growth of *B. cinerea* at a concentration of 5% (w/v) [78]. This may be due to the fact that it changes the plasma membrane, thus inhibiting essential metabolic functions. Some authors have described that high concentrations of sodium benzoate inhibited the growth of *A. Alternata* [80], and this is attributed to the fact that weak acids within the cell create a dissociation, causing that protons and anions accumulate and cannot cross the plasma membrane again [81]. Bicarbonate salts, also were effective to inhibit the growth of various pathogens, the efficiency of this salt is attributed to the fact that it creates cellular

ionic imbalances affecting the synthesis of polyamines and DNA during cell division [82, 83]. With the accessibility of these compounds and their effectiveness, it is possible that they can be adopted to reduce the use of traditional fungal agents. In a study, potassium sorbate was applied at low concentrations (1%) on infected tomato fruits, and significant reduction of disease incidence was obtained against *Rhizoctonia solani*, *Colletotrichum coccodes*, *Botrytis cinerea*, and *Alternaria solani* [84]. In another study, gray mold caused by *Botrytis cinerea* was totally controlled by the application of potassium sorbate at 1% [85]. The effectiveness of potassium sorbate is related to its undissociated form (sorbic acid), which has the ability to penetrate the cell membranes, causing an internal imbalance affecting enzymes related to the growth of microorganisms [86]. In a study on citrus, potassium sorbate at 3% solutions was applied in combination with heat treatment (62°C, 60 s) to evaluate their effectiveness in reducing the disease incidence caused by *Penicillium* strains. The results showed that the treatments can reduce the disease incidence on "Clemenules" (20%), "Nadorcott" mandarins (25%), "Fino" lemon (50%), tangerine "Ortanique" (80%), and "Valencia" oranges (95%) stored 20°C for 7 days. Besides, when infected and treated fruits were stored at 5°C during 60 days, on "Valencia" oranges, the green mold (*Penicillium digitatum*) was reduced up to 95% and blue mold (*Penicillium italicum*) up to 80% [87]. Green mold caused by *P. digitatum* was reduced up to 80% on infected oranges by the application of sodium benzoate (3%) in combination with hot water (53°C) during 60 s, and fruits were stored at 20°C for 7 days [88]. The effectiveness of sodium benzoate is related to its undissociated form of benzoic acid, which can enter the cell membrane, and its neutralization within the cell leads to acidification of the intracellular space, thus affecting the growth of fungus [89]. On the other hand, another alternative to avoid pathogen development is the use of silicates, and the most used in the fruit industry is: potassium, calcium, and sodium. It has been reported that the action of these organic salts causes, among other effects, inhibition of mycelial growth and alteration of the morphology of the hyphae, and in addition, the germination of conidia is inhibited and causes alterations in their external morphology. The application of sodium silicate induces alterations in the cell wall and in the morphology of the hyphae on pathogenic fungi. It is important to mention that the application of silicate as post-harvest treatment presents results similar to those reported with chitosan and tebuconazole [90]. The investigations of the use of inorganic salts in post-harvest stage are not very frequent, even there are not yet many studies that determine the effect that this may have if applied to different types of crops. These salts have been applied on orange [91], melon [92], avocado [93], and papaya [94], with good results due to the treatments, which form a barrier against pathogens on surface fruit. In addition, the application of silicates can improve some quality attributes of fruits like the maintenance of weight and reducing the respiration rate due to the capacity of the silicates to deposit between the cell wall and the cell membrane, thus decreasing the permeability, besides the stomata are covered, maintaining the humidity of the fruit and reducing its respiration [95, 96]. With the accessibility of these compounds and their effectiveness, it is possible that they can be adopted to reduce the use and applications of traditional fungal agents.

5.6 Jasmonic and salicylic acid

Resistance induced to disease in plants by biotic and abiotic elicitors is a very effective method for restricting the spread of fungal infection [97]. In general, pathogen resistance processes in plants are based on their own defense mechanisms, such as pre-existing antimicrobial compounds and inducible defense mechanisms. Resistance to diseases induced in plants and fruits by biotic or abiotic treatments

is a very attractive strategy to control diseases [98]. The signal molecules salicylic acid (SA), jasmonic acid (JA), and methyl jasmonate (MeJA) are endogenous plant growth substances that play key roles in development and responses to environmental stresses. These signal molecules are involved in some signal transduction systems in plants and fruits, which induce particular enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloids, or pathogenesis-related (PR) proteins. This can result in induction of defense responses and provide protection for plants and fruits from pathogen attack [99]. Salicylic acid activates induction of acquired systemic resistance (SAR) response in plants, proving that in the plant-microorganism interaction, the enzyme phenylalanine ammonia lyase (PAL) is induced, which is the key in the biosynthesis of phenolic compounds [100]. Peroxide has an antibiotic activity against pathogens; it could intervene in the signaling cascade for the expression of defense genes. SA regulates activities of enzymes, peroxidase (POD), and polyphenoloxidase (PPO), that are related to induced defense of plants and fruits against biotic and abiotic stress [101]. Jasmonic acid (JA) and methyl jasmonate (MeJA) have been found to occur naturally in a wide range of higher plants. MeJA is an occurring plant growth regulator that modulates many physiological processes including responses to environmental stresses [99]. Studies indicate that acquired systemic resistance depends on signaling mediated by MeJA and is associated with some signal transduction systems, which induce particular enzymes that catalyze biosynthetic reactions to form defense compounds such as polyphenols, alkaloids, reactive oxygen species ($ROS^{\bullet}$), or PR proteins [102]. The exogenous application of MeJA induces and increases the activity of defense enzymes such as β-1,3-glucanase (β-Gluc), chitinase, polyphenoloxidase (PPO), and phenylalanine ammonia lyase (PAL), which are enzymes associated with resistance to diseases [103]. Application of MeJA effectively suppressed gray mold rot caused by Botrytis cinerea in strawberry [104] and decreased fruit decay on papaya fruit infected by *C. gloeosporioides* and *Alternaria alternata* [105]. For grape fruits inoculated with Botrytis cinerea, the application of MJ (0.01 mM) increased the enzymatic activity of PAL and PPO [106], and the same behavior was reported on Hass avocado fruits with an increase in the activity of the resistance enzymes, chitinase, β-1,3-glucanase and PAL [11]. The application of MeJA (10 mM) in cranberry fruit inoculated with *Penicillium citrinum* maintained greater POD and PAL activity [107]. There are several studies on the application of SA in fruits for the induction of defense mechanisms against pathogens. Resistance of the tomato fruit against *Botrytis cinerea* using SA as a resistance inducer, a significant increase in the expression level of the PR1 gene was observed in the fruit and a lower expression in the PR2 and PR3 genes [108]. The post-harvest application of SA (2 mg/mL) showed a decrease in the severity of anthracnose in mango cv. Kensington Pride [109]. Thus, the use of inducers offers several advantages for post-harvest disease control; besides, they can combine it with other methods to enhance their efficacy.

5.7 Coatings and edible film from natural sources

Edible coatings on fruits and films made from natural sources are a novelty method and an alternative to the use of post-harvest chemical treatments, particularly in highly perishable fruits [110]. The coatings act as a barrier during processing, handling and storage, delaying the deterioration of food, improving its quality, and extending their shelf life. The functional properties of edible coatings and films depend on their application and the characteristics of the fruit in which they are going to be used. The nature of the compound that is used to produce the coating strongly influences its efficiency, thus it must take into account both its physical and chemical properties, as well as its mechanical and permeable properties. Based on this, if the edible coatings are used properly, they may be able to delay the ripening of the fruit,

slow the decomposition of the chlorophyll, reduce the weight loss, retain the ascorbic acid, improve the appearance of the fruit, and especially prolong the shelf life [111–113]. To date, the functional properties of different compounds for the production of edible coatings have been studied. Compounds from natural sources such as *Aloe vera* and waxes have shown promising properties to be applied in the preservation of tropical fruits, and therefore coatings based on these compounds can represent an innovation in the commercial application and exploitation of these resources. *Aloe vera* gel is one of the natural compounds, which has gained a great interest. Because of its nature mainly constituted by polysaccharides, it is capable to form a uniform layer on the surface of the fruit and be easily applied [114]. *Aloe vera* coating can improve the post-harvest qualitative and quantitative traits, thus it can be an alternative for chemicals preservative in the commercial storage of tropical and subtropical fruits.

Aloe vera-based coatings have been successfully tested in mango fruit ripening (*Mangifera indica L. cv. Kensington Pride*) [111]. *Aloe vera* coating reduced aroma volatile biosynthesis in the fruit pulp. Likewise, it was found that coatings delayed ripening of the fruit compared to control. They state that this effect was characterized by the suppression respiration and/or delayed climacteric peak, late fruit color development, and a greater firmness in the coated fruit compared to the uncoated ones. Similarly, the effect of edible coatings based on *Aloe vera* to extend the shelf life of the guava (*Psidium guajava*) has been demonstrated. Achipiz et al. [112] found that a coating with a concentration of 4% potato starch and 20% *A. vera* showed a favorable effect by reducing the weight loss and the respiratory rate of the fruit and increasing the firmness and retention of the vitamin C content after 10 days of storage. In another study, the effect of the *Aloe vera* gel coating on the store ability of peach fruits was evaluated [115].

The Carnauba wax stems from the leaves of the Brazilian palm *Copernicia cerifera*. It is produced as a protection method to prevent dehydration and damage. The Carnauba wax as an edible coating is being extensively studied because it has been demonstrated to reduce water loss, improve appearance, and prolong shelf life in a wide variety of fruits. An edible coating based on cassava starch and carnauba wax adding organic acids and calcium chloride was evaluated in mangoes cv Tommy Atkins minimally processed [113]. According to the results, the attributes of sensory, physical, and chemical quality were maintained, and the useful life of fruit was possible to prolong up to 24 days under refrigeration conditions (5 ± 1°C and 90 ± 2% RH).

Saucedo-Pompa et al. [116] developed an edible coating based on candelilla wax to improve avocado quality. Furthermore, they studied the effect of the ellagic acid addition in the shelf life of the fruit. The results showing the application of edible films based on candelilla wax improved the quality of the avocado fruits and extended its shelf life compared to the control fruits. Also, the addition of ellagic acid to the edible film showed an important effect, since it reduced the damage caused by the fungus *C. gloeosporioides* (the main phytopathogenic fungus for avocados) and significantly improved the quality and shelf life of avocado. Another coating based on mesquite gum-candelilla wax was evaluated in Persian limes [117]. The results showed that coatings decreased the weight loss of the fruit. In addition, by adding mineral oil (33%) to the emulsion, they observed that water vapor permeability was significantly improved, as well as its appearance.

5.8 Ultrasound

Ultrasonic is an economically and environmentally viable alternative for the processing of fruit and vegetable post-harvest [118]. Low intensity ultrasound has been used for quality control of fresh fruit and vegetables in pre- and post-harvest processes [119]. Harvesting time and storage period can be indirectly assessed by ultrasound measurements that are linked physicochemical measurements such as

firmness, mealiness, dry weight percentage, oil contents, total soluble solids, and acidity [119]. On the other hand, decontamination of fresh product by ultrasound is relatively recent. The inactivation of microorganism caused by cavitation phenomenon has promoted high intensity ultrasound as method to decontaminate fruits and vegetables. The efficiency of the ultrasound process is affected by several factors such as power level, treatment time, and temperature [120]. Additionally, ultrasound can be applied directly to the medium (water) or in combination with some compounds (organic salts, organic acids, chitosan, among others) to achieve better results. Concerning individual ultrasound application, ultrasound at low frequencies (20 and 40 kHz) has demonstrated to decrease the microbial load of mesophilic aerobes in lettuce (0.9 log CFU/g) and strawberry (1.49 log CFU/g) [121, 122]. At the present time, ultrasound is being implemented in combination with various aqueous sanitizers in order to improve microbial safety and maintain food quality on organic fresh produce. *In vitro* assay, the addition of low weight chitosan (1000 ppm) enhanced the inactivation of *Saccharomyces cerevisiae* by ultrasound (20 kHz) at 45°C in Sabouraud broth (pH 5.6). After 30 min of exposure to chitosan, approximately 1-log cycle reduction of the yeast was obtained leading to a final reduction of more than three log cycles after 30 min of the ultrasonic treatment [123]. In the case of *in vivo* assays, the effectiveness of ultrasound (40 kHz, 5 min) alone and organic acids (0.3, 0.5, 0.7, 1.0, and 2.0% of malic acid, lactic acid, and citric acid) alone and their combination on reducing *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and *Listeria monocytogenes* in fresh lettuce was compared. For all three pathogens, the combined treatment of ultrasound and organic acids resulted in additional 0.8–1.0 log reduction compared to individual treatments, without causing significant quality change (color and texture) on lettuce during 7 day storage. The maximum reductions of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* were 2.75, 3.18, and 2.87 log CFU/g observed after combined treatment with ultrasound and 2% organic acid for 5 min, respectively (Sagong et al., 2011). In peach fruit, the effect of ultrasound (40 kHz, 10 min) and salicylic acid (0.05 mM) either separately, or combined on blue mold caused by *Penicillium expansum* was investigated. The results showed that the application of salicylic acid alone could reduce blue mold, while the use of ultrasound had no effect. Results also revealed that salicylic acid combined with ultrasound treatment was more effective in inhibiting fungal decay during storage than the salicylic acid treatment alone. The combined treatment increased the activities of defense enzymes such as chitinase, β-1,3-glucanase, phenylalanine ammonia lyase, polyphenol oxidase, and peroxidase, which were associated with higher disease resistance induced by the combined treatment. Furthermore, the combined treatment did not impair the quality parameters of peach fruit after 6 days of storage at 20°C [124]. The incorporation of ultrasound alone or in combination with other agents in decontamination process could be a useful preservation technique for post-harvest fruits and vegetables. Combination of ultrasound and sanitizers could increase pathogen reduction without affecting the product quality, while concentration of sanitizers could be reduced as well as treatment time required, saving time and money and avoiding significant risks to consumers.

5.9 Fogging

In order to prolong the shelf life of fruits and vegetables in post-harvest periods, various technologies have been developed that maintain their integrity as well as their nutritional properties. One of the technologies little explored at present is the use of ultrasonic nebulization (Fogging) as a method of distribution of compounds that serve to prevent or control pathogenic diseases in the post-harvest period. Fogging has been used successfully for the spraying of disinfectants such as chlorine dioxide, sodium

hypochlorite, hydrogen peroxide, acetic acid, and ethanol, achieving the control of epiphytic microorganisms on the surface of the strawberry (fungi and bacteria), thus reducing the decay index by up to 83.2%, demonstrating that nebulization is an effective method for the reduction of diseases in the post-harvest stage [125]. Regarding fruit quality, in a study with strawberry, peracetic acid was applied as a disinfectant by ultrasonic nebulization, and the results showed that the anthocyanin and phenolic compound contents were preserved even when the fruits were exposed to low concentrations of peracetic acid [126]. In the post-harvest and fruit storage period, it is necessary to minimize chemical products as environmental precautions and avoid adapting pathogens to various fungicides, causing high losses of between 30 and 50% in vegetables and fruits [125, 127]. The use of ultrasonic nebulization offers advantages such as the reduction of the amount of disinfectant and a better distribution of the treatment on the fruits, and in a study of figs, the inhibition of gray mold disease (B. cinerea) 80% of control was achieved with only the application of chlorine dioxide (1000 μL/L) [127]. Ultrasonic nebulization as a conservation method in the post-harvest period gives high benefits in different ways, by reducing the quantity of substances applied, the exposure time as well as a better distribution of the treatments, its application in fruits and vegetables has not been explored, thus the development of this technology can offer an alternative to the use of chemical fungicides for the control of diseases.

6. Conclusions

Considering the new tendencies in fruit industry and marketing, the use of alternative methods represents a suitable approach for several agriculture commodities not only for controlling post-harvest diseases but also for maintaining fruit quality.

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