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Shelf Life Extension of Fresh Fruit and Vegetables by Chitosan Treatment

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Among alternatives that are currently under investigation to replace the use of synthetic fungicides to control postharvest diseases in fresh produce and to extend their shelf life, chitosan application has shown promising disease control, at both preharvest and postharvest stages. Chitosan shows a dual mode of action, on the pathogen and on the plant, as it reduces the growth of decay-causing fungi and foodborne pathogens and induces resistance responses in the host tissues. Chitosan coating forms a semipermeable film on the surface of fruit and vegetables, thereby delaying the rate of respiration, decreasing weight loss, maintaining the overall quality, and prolonging the shelf life. Moreover, the coating can provide a substrate for incorporation of other functional food additives, such as minerals, vitamins or other drugs or nutraceutical compounds that can be used to enhance the beneficial properties of fresh commodities, or in some cases the antimicrobial activity of chitosan. Chitosan coating has been approved as GRAS substance by USFDA, and its application is safe for the consumer and the environment. This review summarizes the most relevant and recent knowledge in the application of chitosan in postharvest disease control and maintenance of overall fruit and vegetable quality during postharvest storage.

Keywords Edible coating, edible film, induced resistance, foodborne pathogens, antimicrobial activity, postharvest storage

INTRODUCTION

Somewhere between 15% and 50% of fruit and vegetables produced on the global scale is lost after harvest (FAO, 2011), mainly due to microbiological spoilage (Kader, 2005). This percentage is greatly increased in developing countries, where the correct technologies for storage of fruit and vegetable are lacking (FAO, 2011). The susceptibility of fresh produce to postharvest diseases and deterioration of quality attributes increases after harvest and during prolonged storage, as a result of physiological and biochemical changes in the commodities. These changes can favor the development of postharvest pathogens and the incidence of postharvest diseases, which are the major cause of losses through the supply chain. Therefore, the development of decay-control measures that aim to maintain the quality of fruit and vegetables and to provide protection against postharvest diseases after removal from cold storage at the retailer's market shelf will be beneficial to reduce these postharvest losses.

On the other hand, postharvest disease control for fresh horticultural produce should begin at the farm, and this involves the cultural practices and fungicide applications used. The adverse effects of synthetic fungicide residues on human health and the environment, and the possibility of the development of fungicide-resistant pathogens, have led to intensified world-wide research efforts to develop alternative control strategies. In addition, the current consumer trend is more towards 'green' consumerism, with the desire for fewer synthetic additives in food, together with increased safety, excellent nutritional and overall quality, and improved shelf-life. Furthermore, there is the potential for foodborne outbreaks due to contamination of fruit

in the field through dirty irrigation water or treatments, or at postharvest through human handling or improper sanitation (Beuchat, 2002).

Application of chitosan treatment at the preharvest or postharvest stages has been considered as a suitable alternative treatment to replace the use of synthetic fungicides. This can help to prevent postharvest fruit diseases and to extend storage life, while maintaining the overall quality of the different fresh commodities (Bautista-Baños et al., 2006). Chitosan (poly b-(1-4)N-acetyl-d-glucosamine) has been identified as providing an ideal coating, with antimicrobial properties that can induce plant defense responses when applied to vegetal tissues (Devlieghere et al., 2004). On the other hand, chitosan coating also provides a substrate for incorporation of other functional natural food additives, which might improve its antimicrobial properties and prevent deterioration of fruit quality (Vargas et al., 2008). Chitosan treatment in the fresh produce industry is safe for the consumer and the environment, and chitosan has been approved by the United State Food and Drug Administration (USFDA) as a 'Generally Recognized As Safe' (GRAS) food additive (USFDA, 2013).

Nowadays, commercial chitosan formulations are available on the market. Some commercial formulations have been tested for the control of postharvest diseases in different fresh produce commodities, as shown in Table 1. The commercial formulations used in plant disease management, not only for the control of postharvest decay of fruit, include: Chitogel (Ecobulle, France) (Ait Barka et al., 2004; Elmer and Reglinski, 2006); Biochikol 020 PC (Gumitex, Lowics, Poland) (Nawrocki, 2006); Armour-Zen (Botry-Zen Limited, Dunedin, New Zealand) (Reglinski et al., 2010); Elexa 4 Plant Defense Booster (Plant Defense Booster Inc., USA) (Elmer and Reglinski, 2006); and Kendal Cops (Iriti et al., 2011). The main differences between practical

grade chitosan solutions and commercial chitosan formulations arise from the techniques used for their preparation and application, which is more immediate for the commercial formulations. Indeed, while practical grade chitosan needs to be dissolved in an acid medium some hours before use, the commercial formulations can be quickly dissolved in water (Romanazzi et al., 2013). However, nowadays, chitosan-based formulations used either at preharvest or postharvest are not registered as plant protectant products, but as growth adjuvants.

The aim of this review is to summarize the most recent published and relevant advances in the application of chitosan for fresh horticultural produce, in terms of postharvest disease control, maintenance of overall product quality, use as a health promoting compound, and food safety issues. For better clarity, the data obtained for the *in vivo* applications of chitosan are divided into sections that consider temperate fruit, tropical fruit, and vegetables, as the environment and the way of cultivation differ across these categories.

INFLUENCE OF PREHARVEST CHITOSAN APPLICATION OF ON POSTHARVEST DISEASE CONTROL

Although many studies have reported on the effectiveness of chitosan treatments at the postharvest stage, the research findings on the evaluation of the preharvest application of chitosan on the control of postharvest decay in fresh produce is limited (Tables 2-4). However, chitosan applications prior to harvest might be suitable for fruit, such as table grapes and strawberries, because these fruit have a bloom on the surface and/or can suffer postharvest wetting or handling. Moreover, preharvest treatment can provide a

115 preventive effect against pathogens, as the development of postharvest disease often
116 arises from an inoculum that survives and accumulates on the fruit surface in the field or
117 in the packaging line after the harvest.

118 Table grape bunches sprayed in the field with solutions of practical grade
119 chitosan at three different concentrations (1%, 0.5%, 0.1%), as once (21 days) or twice
120 (21, 5 days) before harvest, showed significantly reduced gray mold infections caused
121 by *Botrytis cinerea* after 30 days storage at 0 °C, followed by 4 days of market shelf life
122 (Romanazzi et al., 2002). Chitosan treatment showed postharvest disease control that is
123 as effective as procymidone field treatment and SO_2 fumigation of grapes after low
124 temperature storage (Romanazzi et al., 2002). Berries sprayed with chitosan preharvest
125 have shown decreased incidence and severity of gray mold in artificially inoculated
126 fruits, with the best control of gray mold obtained 1-2 days after the application
127 (Romanazzi et al., 2006). Postharvest disease has also been reduced by preharvest
128 chitosan treatment and by postharvest UV-C irradiation (0.36 J/cm^2 for 5 min), with the
129 combination of these treatments providing a synergistic interaction (Romanazzi et al.,
130 2006). Application of the antagonistic fungus *Cryptococcus laurentii* combined with 1%
131 chitosan on the day before harvest significantly reduced natural decay in table grapes
132 stored at 0 °C for 42 days, and thereafter held at 20 °C for 3 days under market-
133 simulation conditions (Meng et al., 2010b). In another study, three different commercial
134 formulations containing chitosan (Armour-Zen, OII-YS, Chito Plant) were compared in
135 a field trial in which they were applied four times during the development of
136 'Thompson Seedless' grapes (berry set, pre-bunch closure, veraison, and 2 weeks before
137 harvest). The natural incidence of postharvest gray mold after storage at 2 °C for 5
138 weeks was reduced by the chitosan, regardless of the commercial formulation used

among the three that were tested. Other rot diseases that were mainly caused by *Alternaria* spp. and *Penicillium* spp. were mainly reduced by the OII-YS chitosan formulation, which was even more effective than the fungicide program (Feliziani et al., 2013a).

Strawberries sprayed with chitosan at full bloom or at the green-fruit or whitening fruit stages have shown decreased incidence of gray mold and Rhizopus rot infections using natural inocula of *B. cinerea* and *Rhizopus stolonifer*, as seen after 10 days of storage at 0 °C followed by 4 days under market-simulation conditions. The disease control with 1% chitosan was more effective than the currently used chemical fungicides: procymidone (40 g hl^{-1} a.i.) used at the full bloom and green fruit stages; and pyrimethanil used at the whitening fruit stage (Romanazzi et al., 2000). Preharvest treatments with 1% and 2% chitosan decreased the incidence of postharvest gray mold from a natural inoculum, and after preharvest and postharvest inoculation, these applications performed significantly better than a fungicide. This treatment with 1% chitosan also performed better than that with 2% chitosan, which was occasionally phytotoxic (Mazaro et al., 2008). Preharvest spraying with 0.2%, 0.4% and 0.6% chitosan decreased postharvest gray mold and maintained the kept quality of strawberries during storage at 3 °C and 13 °C. Here, the incidence of disease decreased with increased chitosan concentration (Reddy et al., 2000a).

Sweet cherries treated 7 days before harvest date with 0.1%, 0.5% and 1% chitosan showed decreased incidence of gray mold and brown rot after 2 weeks of storage at 0 °C followed by 7 days of shelf life, as compared to the untreated controls (Romanazzi et al., 1999). At the highest chitosan concentration (1%), the disease reduction was not different with respect to that seen after application of tebuconazole.

Similar results were obtained when 1% chitosan was applied 3 days before harvest, as it reduced the incidence of postharvest disease in sweet cherries to the same level as the commercially applied synthetic fungicide fenhexamid (Feliziani et al., 2013b). Chitosan (1%) application 7 days before harvest and postharvest hypobaric treatments at 0.25 atm or 0.50 atm for 4 h showed synergistic effects in the control of total rot diseases in sweet cherries stored at 0 °C for 14 days, and thereafter held at 20 °C for a 7-day shelf life (Romanazzi et al., 2003).

Fornes et al. (2005) reported that 'Clemenules' mandarin fruit treated 86 days before harvest and at a postharvest stage with low concentrations of chitosan (0.0125% to 0.125%) showed reduced water-spot incidence associated with fruit senescence. All of these treatments reduced the number of injured fruit, and the best results were achieved with the highest chitosan concentration (0.125%), which reduced water spot incidence by 52%.

EFFECT OF POSTHARVEST CHITOSAN APPLICATION ON DISEASE CONTROL

Use of chitosan for postharvest disease control in temperate fruit was investigated in the 1990s in many studies. These studies concerned the application of chitosan in general or focused in a group of chitosans, such as oligochitosan that are characterized by low molecular weight. El Ghaouth et al. (1991a; 1992a) and Zhang and Quantick (1998) reported that the control of gray mold and Rhizopus rot in chitosan-coated strawberries was similar to synthetic fungicide application. *Cladosporium* spp. and *Rhizopus* spp. infections were also reported to decrease in artificially inoculated strawberry fruit coated with chitosan and stored at 4 °C to 6 °C for 20 days (Park et al., 2005). Similar

results were obtained for table grapes, as small bunches dipped in 0.5% and 1% chitosan solutions, and thereafter artificially inoculated with a *B. cinerea* conidial suspension (by spraying), and stored at low (0 °C) or room (20 °C) temperatures. The chitosan treatment decreased the spread of gray mold infection from one berry to the other berries (nesting) (Romanazzi et al., 2002). Li and Yu (2001) reported that 0.5% and 0.1% chitosan significantly reduced the incidence of brown rot caused by *Monilinia fructicola* in peach stored at 23 °C, compared to the untreated fruit. Similarly, application of 1% chitosan reduced postharvest diseases of sweet cherry (Feliziani et al., 2013b). Treatments with chitosan and oligochitosan reduced disease incidence caused by *Alternaria kikuchiana* and *Physalospora piricola* and inhibited lesion expansion of the pear fruit stored at 25 °C. These disease-control effects of chitosan and oligochitosan were concentration dependent and weakened over the incubation time. Indeed, at the lowest chitosan concentration, its effectiveness was the lowest for disease control especially after 5 days of storage at ambient temperatures, compared to the beginning of storage (Meng et al., 2010a). For vegetables such as tomatoes, the infection diameter caused by *R. stolonifer* was 15% less than for the control when treated with chitosan at 1.0%, 1.5% and 2.0%, regardless of the molecular weight (Bautista-Baños and Bravo-Luna, 2004).

The recent advances concerning chitosan application on postharvest temperate fruit have aimed to combine the biopolymer with other alternatives to fungicides, such as decontaminating agents, plant extracts, essential oils, biocontrol agents, or physical treatments, to provide improved synergistic interactions for the control of postharvest diseases, compared to chitosan alone.

Chitosan has been applied in combination with various biocontrol agents, such as *Candida satoiana* or *Cryptococcus laurentii*, which are microorganisms that have antagonistic actions against postharvest pathogens (El-Ghaouth et al., 2000; De Capdeville et al., 2002; Yu et al., 2007; 2012; Meng et al., 2010b). Spraying of the antagonistic yeast, *C. laurentii*, followed by postharvest chitosan coating significantly reduced the natural decay of table grapes stored at 0 °C. The chitosan coating enhanced the effectiveness of the preharvest spray (Meng et al., 2010b). *C. laurentii* associated with 0.5% chitosan and calcium chloride was effective for the reduction of postharvest blue mold caused by *Penicillium expansum* in pear as well. This combination resulted in more effective mold control than chitosan or *C. laurentii* alone, although chitosan at 0.5% inhibited the growth of the biocontrol yeast *in vitro* and *in vivo*. Moreover, after 6 days of incubation, the combined treatment with *C. laurentii*, chitosan and calcium chloride inhibited mold decay by nearly 89%, which was significantly higher than the treatments with *C. laurentii*, chitosan or calcium chloride alone, and with the combinations of *C. laurentii* and chitosan, and *C. laurentii* and calcium chloride (Yu et al., 2012). The combination of chitosan and *C. laurentii* on apple resulted in synergistic inhibition of blue mold rot, which was the most effective treatment at the optimal concentration of 0.1% chitosan (Yu et al., 2007). In tropical fruit, the application of the bacterium *Lactobacillus plantarum* alone or in combination with 2% chitosan preserved the quality characteristics of rambutan fruit (Martínez-Castellanos et al., 2009). Similarly, the combination of *Candida saitoana* with 0.2% glycolchitosan was more effective in controlling gray and blue mold of apple and green mold caused by *Penicillium digitatum* of oranges and lemons than the yeast or glycolchitosan alone (El-Ghaouth et al., 2000). On the contrary, the combination of chitosan with *C. saitoana* or

234 with UV-C had no synergistic effects on the progress of blue mold of apple, although a
235 single treatment provided significant reductions (De Capdeville et al., 2002).

236 Extracts obtained from many plants have recently gained popularity and
237 scientific interest for their antimicrobial properties, and thus their activities against
238 decay-causing fungi on fruit and vegetables have been investigated (Gatto et al., 2011).
239 Chitosan coating can be used as a carrier to incorporate plant essential oils or extracts
240 that have antifungal activities or neutraceutical properties. Chitosan incorporated with
241 limonene, a major component of lemon essential oils, which has also been given the
242 GRAS status by the USFDA, promoted the preservation of strawberry fruit during their
243 shelf life (Vu et al., 2011). The addition of lemon essential oils enhanced chitosan
244 antifungal activities both in *in in-vitro* tests and during cold storage of strawberries
245 inoculated with a spore suspension of *B. cinerea* (Perdones et al., 2012). On table
246 grapes, the combination of 1% chitosan and a grapefruit seed extract improved decay
247 control with respect to single applications of chitosan and maintained the quality of
248 table grapes (Xu et al., 2007b). Similarly, chitosan coatings that contained bergamot oil
249 or cinnamon oil improved the quality of stored table grapes (Sánchez-González et al.,
250 2011) and of sweet peppers (Xing et al., 2011a), respectively. Chitosan coating without
251 or with essential oils (bergamot, thyme and tea-tree oil) was applied to oranges as
252 preventive or curative treatments against blue mold. In all cases, the addition of the
253 essential oils improved the antimicrobial activities of chitosan; however, the preventive
254 and curative antimicrobial treatments with coatings containing tea-tree oil and thyme,
255 respectively, were the most effective in the reduction of the microbial growth, as
256 compared to the uncoated samples (Cháfer et al., 2012). On the other hand, in another
257 study, combinations of cinnamon extract and chitosan were not compatible, as the

cinnamon extract reduced the effectiveness of chitosan in the control of banana crown rot caused by a fungal complex, *Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae* and in delaying fruit senescence during storage (Win et al., 2007). Treatments of papaya with 0.5% or 1.5% chitosan, or with the combination of 1.5% chitosan with an aqueous extract of papaya seed, controlled the development of anthracnose diseases of fruit inoculated with *Colletotrichum gloeosporioides*. However, no synergistic effects were obtained with the combination of chitosan at 1.5% and the aqueous extract of papaya for the control of the fungal growth (Bautista-Baños et al., 2003). Similarly, limited control of *R. stolonifer* was observed for chitosan-coated tomatoes in combination with beeswax and lime essential oils (Ramos-García et al., 2012).

In some trials chitosan was combined with oleic acid. Coatings based on chitosan either without or with oleic acid at different percentages delayed the appearance of natural fungal infections in comparison to uncoated strawberries. When oleic acid was added to the chitosan coating, there were fewer signs of fungal infection during strawberry storage, especially when the coatings contained the higher levels of oleic acid, which enhanced the antimicrobial properties of chitosan (Vargas et al., 2006).

The postharvest application of chitosan has been combined with physical means for the control of postharvest decay of fruit and vegetables, such as UV-C irradiation, hypobaric treatment, and heat curing. Shao et al. (2012) studied the effects of heat-treatment at 38 °C for 4 days before and after coating apples with 1% chitosan. As well as complete control of blue mold and gray mold on these artificially inoculated apples during storage, chitosan coating followed by heat treatment improved the quality of the

stored fruit. Moreover, the presence of chitosan coating prevented the occurrence of heat damage on the fruit surface (Shao et al., 2012). In another investigation, the development of postharvest brown rot on peaches and nectarines was controlled through the heating of fruit to 50 °C for 2 h under 85% relative humidity, which eradicated pre-existing *Monilinia* spp. infections that came from the field, with the application of 1% chitosan at 20 °C then protecting the fruit during handling in the packaging houses and until consumer use (Casals et al., 2012). The combination of immersion in hot water (46.1 °C for 90 min) and in 2% chitosan was beneficial to the storage qualities of mango, compared to untreated mangoes or to fruit treated only with hot water or chitosan (Salvador-Figueroa et al., 2011). Sweet cherries dipped in 1% chitosan and exposed soon after to hypobaric treatment (0.50 atm for 4 h) showed significant reductions in postharvest natural brown rot, gray mold, and total rot diseases, in comparison with the control and with each treatment applied alone. This combination produced a synergistic effect in its reduction of brown rot and total rots (Romanazzi et al., 2003). Chitosan was also applied as a technology to improve benefits obtained with modified atmosphere packaging. The combination of chitosan coating and modified atmosphere packaging was effective in preventing decay and browning, and in retaining the pericarp color in litchi fruit (De Reuck et al., 2009).

To improve its efficacy in controlling postharvest decay of fruit and vegetables, chitosan has been combined with decontaminating agents. The combination of 0.5% chitosan with 10% or 20% ethanol, which is commonly used in the food industry for its antifungal properties, improved decay control with respect to the single treatments in *B. cinerea*-inoculated table grapes, as single berries or as clusters (Romanazzi et al., 2007). Application of natamycin, which is a common food additive that is used against mold

and yeast growth, in combination with a bilayer coating that contained chitosan and polyethylene wax microemulsion, extended the shelf life of Hami melon, with decreases in weight loss and decay (Cong et al., 2007). Chitosan alone or in combination with sodium bicarbonate or ammonium carbonate significantly reduced the severity of anthracnose for both inoculated and naturally infected papaya fruit. The effects of chitosan combined with ammonium carbonate on the incidence and severity of anthracnose was greater than chitosan alone, and than chitosan with sodium bicarbonate (Sivakumar et al., 2005b). Similarly, the combination of chitosan with potassium metabisulfite was tested in litchi fruit. Both chitosan and the combination of chitosan and potassium metabisulfite decreased postharvest decay of these litchi fruit (Sivakumar et al., 2005a).

It is also worth mentioning the combination of chitosan with arabic gum, which is a common polysaccharide that is frequently used as an additive in the food industry; this combination controlled banana anthracnose caused by *C. musae* both *in vitro* and *in vivo*, and it enhanced the shelf-life of banana fruit (Maqbool et al., 2010a; 2010b).

In some other studies the most suitable acids were tested for the dissolving of chitosan powder, and it was shown that practical grade chitosan should be dissolved in an acid solution to activate its antimicrobial and eliciting properties. Chitosan dissolved in 10 different acids (as 1% solutions of acetic, L-ascorbic, formic, L-glutamic, hydrochloric, lactic, maleic, malic, phosphoric, and succinic acids) was effective in reducing gray mold incidence on single table grape berries (Romanazzi et al., 2009). However, the greatest reduction of gray mold (about 70%, compared with the control) was observed after immersion of the berries in chitosan dissolved in acetic acid or formic acid, whereas there was intermediate effectiveness with chitosan dissolved in

hydrochloric, lactic, L-glutamic, phosphoric, succinic, and L-ascorbic acids. The least effective treatments were chitosan dissolved in maleic or malic acids (Romanazzi et al., 2009).

MODE OF ACTION OF CHITOSAN AGAINST THE POSTHARVEST PATHOGENS

Due to the wide range of antifungal activities against postharvest pathogens (Table 5), chitosan coating can be applied as a biocoating to prolong the postharvest life of fresh produce (Bautista-Baños et al., 2006).

The antimicrobial activities of chitosan appear to rely on electrostatic interactions between positive chitosan charges and the negatively charged phospholipids in the fungal plasma membrane. Chitosan first binds to the target membrane surface and covers it, and in a second step, after a threshold concentration has been reached, chitosan causes membrane permeabilization and the release of the cell contents (Palma-Guerrero et al., 2010). There are usually low levels of Ca^{2+} in fungi cytosol, due to the barrier formed by the plasma membrane, which has hermetic seals that regulate the passage of Ca^{2+} gradients. This process also involves the homeostatic mechanism, where the Ca^{2+} concentration regulates itself within the cytosol, and it sends the excess Ca^{2+} out of the cell or stores it in the intracellular organelles. Thus, as chitosan is applied, the homeostatic mechanism becomes drastically transformed, because as it forms channels in the membrane, it allows the free passage of Ca^{2+} down its gradients, which cause instabilities in the cells that can lead to death of the cell itself (Palma-Guerrero et al., 2009). In addition, inhibitory effects of chitosan on the H^{+}-ATPase in the plasma membrane of *R. stolonifer* has been reported. García-Rincón et al. (2010)

suggested that the decrease in H^+-ATPase activity can induce the accumulation of protons inside the cell, which would result in inhibition of the chemiosmotic driven transport that allows H^+/K^+ exchange. Moreover, a rapid efflux of potassium from cells of *R. stolonifer* has been reported as an effect of chitosan treatment; this was combined with an increase in pH of the culture medium, which was chitosan-concentration dependent. Both of these phenomena were related to the leaking of internal cellular metabolites (García-Rincón et al., 2010). Similarly, when *R. stolonifer* was grown in media containing chitosan, the release of proteins by the fungal cells increased significantly. It was proposed that this release of proteins from the cell to the supernatant is because there are sites where the cell membrane is damaged by chitosan (Guerra-Sánchez et al., 2009).

Besides its capacity for membrane permeabilization, chitosan can also penetrate into fungal cells. Fluorescent labeled chitosan was detected in fungal conidia and it was hypothesized that chitosan itself permeabilizes the plasma membrane to allow its entry into the cytoplasm (Palma-Guerrero et al., 2008; 2009). Another study used fluorescence visualization to demonstrate that oligochitosan can penetrate the cell membrane of *Phytophthora capsici*, and that, as it is positively changed, chitosan can bind to intracellular targets, such as DNA and RNA, which are negatively charged (Xu et al., 2007a). Similarly, observations made on *Aspergillus niger* have revealed the presence of labeled chitosan both inside and outside the cells, and the permeated chitosan was suggested to block DNA transcription, and therefore to inhibit the growth of the fungus (Li et al., 2008).

Several studies have described the morphological changes on fungal hyphae and reproductive structures that can be induced by chitosan. Scanning electron microscopy

observations of *Fusarium sulphureum* treated with chitosan have revealed effects on hypha morphology. The growth of hyphae treated with chitosan was strongly inhibited, and they were tightly twisted and formed rope-like structures. Spherical or club-shaped abnormally inflated ends were observed on the twisted hyphae, which were swollen and showed excessive branching. Further transmission electron microscopy observations have indicated ultrastructural alterations of the hyphae by chitosan. These changes included cell membrane disorganization, cell-wall disruption, abnormal distribution of the cytoplasm, non-membranous inclusion bodies in the cytoplasm, considerable thickening of the hyphal cell walls, and very frequent septation with malformed septa (Li et al., 2009). Examination of ultrasections of the hyphae and conidia of chitosan-treated *Alternaria alternata* revealed marked alterations to the cell wall. The chitosan-treated mycelia showed predominantly loosened cell walls, and in some areas, there was also lysis. The conidia exposed to chitosan were intensely damaged, and usually eroded, with broken cell walls seen that contained in some cases no cytoplasm (Sánchez-Domínguez et al., 2011). *R. stolonifer* subjected to the formulation of chitosan with beeswax and lime essential oils showed no development of the typical reproductive structures, and its mycelia were distorted and swollen (Ramos-García et al., 2012). In another investigation, chitosan-treated spores of *R. stolonifer* showed numerous and deeper ridge formations that were not observed on non-treated spores (Hernández-Lauzardo et al., 2008). Chitosan induced morphological changes of the mycelia of *B. cinerea* and *R. stolonifer* that were characterized by excessive hyphal branching, as compared to the control (El Ghaouth et al., 1992a). This was confirmed in another study, in which there was the induction of marked morphological changes and severe structural alterations in chitosan-treated cells of *B. cinerea*. Microscopic observations

showed coagulation in the fungus cytoplasm that was characterized by the appearance of small vesicles in the mycelia treated with chitosan. In other cases, the mycelia contained larger vesicles, or even empty cells, which were devoid of cytoplasm (Ait Barka et al., 2004). The area and the elliptical form of the spores was significantly different when *C. gloeosporioides* was grown on potato dextrose agar with added chitosan, compared to potato dextrose agar alone (Bautista-Baños et al., 2003). Similarly, the hyphal and germ-tube morphology of *C. gloeosporioides* growing on chitosan showed malformed hyphal tips with thickened walls. Many swellings occurred in the hyphae or at their tips, whereas in the controls cells the walls and germ tubes were smooth with no swellings or vacuolation (Ali and Mahmud, 2008; Ali et al., 2010). The scanning electron micrographs showed normal growth of hyphae in the untreated controls for *C. gloeosporioides*, whereas there was hyphal agglomeration and formation of large vesicles in the mycelia in samples treated with chitosan-loaded nanoemulsions (Zahid et al., 2012). The fungal mycelia of *Sclerotinia sclerotiorum* exposed to chitosan were deformed, twisted and branched, or indeed, dead, with no visible cytoplasm in the fungal cells, whereas the untreated mycelia were normal in appearance (Cheah et al., 1997).

Not all fungi show the same sensitivity to chitosan, which might be due their intrinsic characteristics. New findings relating to the permeabilization of the plasma membrane of different cell types of the fungi *Neurospora crassa* and the membrane composition among various resistant and non-resistant chitosan fungi appear to provide important factors (Palma-Guerrero et al., 2008; 2009; 2010). By imaging fluorescently labeled chitosan using confocal microscopy, it was seen that chitosan binds to the conidial surfaces of all of the species tested, although it only consistently permeabilized

426 the plasma membranes of some of the fungi. Some of the other fungi formed a barrier to
427 the chitosan. Analysis of the main plasma membrane components revealed important
428 differences in the fatty acid compositions between the chitosan-sensitive and chitosan-
429 resistant fungi. The cell membranes of chitosan-sensitive fungi showed higher content
430 of the polyunsaturated fatty acid linolenic acid, higher unsaturation index, and lower
431 plasma membrane fluidity. Chitosan binding should induce an increase in membrane
432 rigidity in the regions to which it attaches. This interaction will enhance the differences
433 in fluidity between the different membrane regions, which can cause membrane
434 permeabilization. In a saturated, more rigid membrane, the changes in rigidity induced
435 by chitosan binding would be much lower, with little permeabilization, even in the
436 presence of negatively charged phospholipid headgroups (Palma-Guerrero et al., 2010).

437 The antifungal activities of chitosan have been reported to vary according to its
438 molecular weight and concentration. It has also been noted that, in general, fungal
439 growth inhibition increases as the concentration of chitosan increases in the cases of *B.*
440 *cinerea* (El Ghaouth et al., 1992a; 2000; Ben-Shalom et al., 2003; Chien and Chou,
441 2006; Liu et al., 2007), *R. stolonifer* (El Ghaouth et al., 1992a), *Penicillium citrinum*
442 (Xing et al., 2011b); *P. digitatum* (Chien and Chou, 2006), *Penicillium italicum* (Chien
443 and Chou, 2006), *P. expansum* (El Ghaouth et al., 2000; Liu et al., 2007; Yu et al.,
444 2007), *M. fructicola* (Yang et al., 2010; 2012), *Botrydiplodia lecanidion* (Chien and
445 Chou, 2006), *C. gloeosporioides* (Jitareerat et al., 2007; Muñoz et al., 2009; Ali and
446 Mahmud, 2008; Abd-Alla and Haggar, 2010; Ali et al., 2010), *Fusarium solani* (Eweis
447 et al., 2006), *A. kikuchiana* (Meng et al., 2010a) and *P. piricola* (Meng et al., 2010a),
448 although it decreases in the case of *A. niger* (Li et al., 2008). In some studies, the
449 antifungal activity of chitosan decreased with an increase in molecular weight, within

the range of 50 kDa to 1000 kDa (Li et al., 2008). The highest inhibitory effect against the growth of *R. stolonifer* was observed with low molecular weight chitosan, while the high molecular weight chitosan showed a greater effect on the development of the spores (Hernández-Lauzardo et al., 2008). High molecular-weight chitosan had the lowest inhibitory effects on *B. cinerea* growth, compared to the low molecular weight chitosan (Badawy and Rabea, 2009). In the case of *S. sclerotiorum*, there was a negative correlation between mycelial growth inhibition and chitosan molecular weight (Ojaghian et al., 2013). Spore germination and germ-tube elongation of *A. kikuchiana* and *P. piricola* were significantly inhibited by chitosan and oligochitosan, although when compared to chitosan, oligochitosan was more effective for the inhibition of spore germination (Meng et al., 2010a). However, other investigations have shown fungal growth inhibition by chitosan, regardless of the type of chitosan (Chien and Chou, 2006), without any fungicidal or fungistatic patterns among low, medium, and high molecular weight chitosans tested with different isolates of *C. gloeosporioides* (Bautista-Baños et al., 2005) and *R. stolonifer* (Guerra-Sánchez et al., 2009).

INDUCTION OF RESISTANCE BY CHITOSAN IN FRUIT TISSUES

Plant resistance towards pathogens occurs through hypersensitive responses that result in cell death at the penetration site, structural alterations, accumulation of reactive oxygen species (ROS), synthesis of secondary metabolites and defense molecules, and activation of pathogenesis-related (PR) proteins (Van-Loon and Van-Strien, 1999). The application of external elicitors to vegetative tissue can trigger plant resistance, by simulating the presence of a pathogen. Several studies have reported that chitosan can

induce a series of enzyme activities and the production of various compounds that are correlated with plant defense reactions to pathogen attack (Bautista-Baños et al., 2006) (Tables 6-8).

Chitosan can increase PR gene function through multiple modes, which includes activation of cell surface or membrane receptors, and internal effects on the plant DNA conformation, which can, in turn, influence gene transcription (Hadwiger, 1999). Histochemical staining of chitosan polymers indicates that chitosan accumulates in the plant cell wall, cytoplasm, and nucleus. The accumulation of positively charged chitosan along with its high affinity for negatively charged DNA suggests that it has a direct effect on the regulation of plant defense responses, with influences on mRNA and protein synthesis (Hadwiger and Loschke, 1981).

Phenylalanine ammonia lyase (PAL) is the key enzyme in the phenol synthesis pathway (Cheng and Breen, 1991), and the accumulation of phenols that act as phytoalexins is considered the primary inducible response in plants against a number of biotic and abiotic stresses (Bhattacharya et al., 2010). Chitosan application has been reported to increase PAL activity in treated fruit tissue. Table grape bunches with preharvest spraying with chitosan showed a three-fold increase in PAL activity in the berry skin 24 h and 48 h after chitosan application (Romanazzi et al., 2002). PAL elicitation by chitosan was confirmed with table grapes sprayed in the vineyard without or with *C. laurentii* and coated with chitosan postharvest, and then stored at 0 °C (Meng et al., 2008; 2010b; Meng and Tian, 2009). Chitosan treatments induced the activity of PAL in sweet cherry (Dang et al., 2010) and strawberry (Romanazzi et al., 2000; Landi et al., 2014), thus enhancing the fruit defense responses.

Chitinase and β-1,3-glucanase are two PR proteins that participate in defense against pathogens, as these can partially degrade the fungal cell wall (Van-Loon and Van-Strien, 1999). Increases in the activities of chitinase and β-1,3-glucanase were demonstrated as a result of chitosan application in 'Valencia' oranges, 24 h after the chitosan treatment. It was proposed that these changes in the enzyme activities might have contributed to the reduction of black spot in the orange fruit (Canale Rappussi et al., 2009). Similarly, chitosan coating significantly reduced the decay of strawberry and raspberry, and induced a significant increase in chitinase and β-1,3-glucanase activities of the berries, as compared to the controls (Zhang and Quantick, 1998; Landi et al., 2014). Compared to the untreated fruit, the high chitinase and β-1,3-glucanase activities in chitosan-treated strawberries reinforced the microbial defense mechanism of the fruit and accentuated the resistance against fungal invasion (Zhang and Quantick, 1998; Wang and Gao, 2012). The chitinase and β-1,3-glucanase activities of papaya and mango subjected to chitosan treatment were much higher than in the untreated fruit (Jitareerat et al., 2007; Hewajulige et al., 2009), and oligochitosan treatment significantly enhanced the activities of chitinase and β-1,3-glucanase in pear fruit (Meng et al., 2010a). In table grapes, preharvest chitosan treatments from three different commercial formulations induced the activity of endochitinase, while two of the chitosan formulations induced exochitinase activity (Feliziani et al., 2013a).

In fruit tissue, the high activity of pectic enzymes, such as polygalacturonase, cellulase and pectate lyase, was shown to be closely associated with the weakening of the plant cell wall, thus resulted in softening of the fruit and greater susceptibility to storage rots (Stevens et al., 2004). Down-regulation of polygalacturonase resulted in firmer fruit (Atkinson et al., 2012). In peach fruit, the chitosan treatments somewhat

inhibited polygalacturonase activity throughout the storage period. In particular, the combination consisted of a coating of chitosan and calcium chloride, the polyethylene packaging, and intermittent warming, with markedly inhibited polygalacturonase activity at the end of the refrigerated storage (Ruoyi et al., 2005). The macerating enzyme activities in tomato tissue, such as polygalacturonase, pectate lyase, and cellulose, in the vicinity of lesions caused by the pathogen *A. alternata* were less than half in chitosan-treated fruit, compared with untreated fruit. Chitosan inhibited the development of black mold rot of tomatoes and reduced the production of pathogenic factors by the fungus (Reddy et al., 2000b).

Chitosan treatment might induce fruit disease resistance through regulation of ROS levels, antioxidant enzymes, and the ascorbate–glutathione cycle. ROS, such as H_2O_2 and O_2^-, are the earliest events that correlate plant resistance to pathogens (Baker and Orlandi, 1995); these are involved in the development of disease resistance in fruit (Torres et al., 2003). Although ROS might contribute to an enhancement of the plant defense, high level of ROS can cause lipid peroxidation and lead to the loss of membrane integrity of plant organs. To prevent harmful effects of excess ROS on plant tissues, the ROS can be detoxified by an antioxidant system. This consists of non-enzymatic antioxidants, such as ascorbic acid, glutathione, and phenolic compounds, and antioxidant enzymes, such as superoxide dismutase, peroxidases and catalases. Chitosan application was reported to reduce ROS in tissues of treated fruit, such as pear (Li et al., 2010a) and guava (Hong et al., 2012), and to lower the hydrogen peroxide content in litchi (Sun et al., 2010), pear (Li et al., 2010a), table grapes (Feliziani et al., 2013a) and strawberry (Romanazzi et al., 2013). This might be due to direct effects, as

chitosan itself has antioxidant activity and scavenges hydroxyl radicals (Yen et al., 2008), or to indirect effects, as chitosan induces the plant antioxidant system.

Higher levels of glutathione were reported after chitosan treatment in litchi (Sun et al., 2010), strawberry (Wang and Gao, 2012) and orange (Zeng et al., 2010). Higher quantities of ascorbic acid have also been reported after chitosan treatments in fruit tissues of strawberry (Wang and Gao, 2012), peach (Li and Yu, 2001; Ruoyi et al., 2005), sweet cherry (Dang et al., 2010; Kerch et al., 2011), jujube (Qiuping and Wenshui, 2007; Xing et al., 2011b), orange (Zeng et al., 2010), citrus (Chien and Chou, 2006), longan (Jiang and Li, 2001), guava (Hong et al., 2012), mango (Jitareerat et al., 2007; Zhu et al., 2008) and litchi (Sun et al., 2010). The reduction of ascorbic acid loss in chitosan-coated sweet cherries was proposed to be due to the low oxygen permeability of the chitosan coating around the fruit surface, which lowers the oxygen level and reduces the activity of the ascorbic acid oxidase enzymes, which prevents the oxidation of ascorbic acid (Dang et al., 2010).

The presence of antioxidants, such as the phenols, can substantially reduce the ROS content of plant tissues, as their hydroxyl groups and unsaturated double bonds make them very susceptible to oxidation (Rice-Evans et al., 1997). Moreover, phenolic compounds are involved in plant responses against biotic and abiotic stresses (Lattanzio et al., 2006; Bhattacharya et al., 2010). Chitosan coating was effective in the intensification of total antioxidant capacity of treated apricot, with increases in the phenolic compounds in the fruit tissue (Ghasemnezhad et al., 2010). In tomato, the content of phenolic compounds increased in chitosan-treated fruit compared to the untreated fruit (Liu et al., 2007), and this increase was directly proportional to the chitosan concentration used (Badawy and Rabea, 2009). Table grapes treated with

chitosan had higher phenolic compound contents (Shiri et al., 2012; Feliziani et al., 2013a). Anthocyanin, flavonoid and total phenolics contents of chitosan treated litchi decreased more slowly than in untreated fruit (Zhang and Quantick, 1997; Jiang et al., 2005; De Reuck et al., 2009). Kerch et al. (2011) reported that total phenols and anthocyanin content increased in chitosan-treated sweet cherry after 1 week of cold storage, while their contents decreased in chitosan-treated strawberry stored under the same conditions. Similarly, in strawberry, chitosan-coated fruit had lower anthocyanin content, as the anthocyanins were synthesized at a slower rate than for the non-treated berries (El Ghaouth et al., 1991a), and the rate of pigment development was lower with an increase in chitosan concentration (Reddy et al., 2000a). The anthocyanin contents significantly decreased throughout storage in strawberries coated with chitosan combined with oleic acid, whereas no significant changes were seen in the control samples at the end of the storage (Vargas et al., 2006). On the contrary, Wang and Gao (2012) reported that strawberries treated with chitosan maintained better fruit quality, with higher levels of phenolics, anthocyanins and flavonoids. In another study, the application of chitosan to strawberry increased the expression of genes involved in the biosynthesis of flavonoid compounds, such as chalcone isomerase, flavonol synthase, anthocyanidin synthase (Landi et al., 2014). Several factors, such as the cultivar of the studied commodity, the stage of maturation, the storage conditions, could account to explain the different responses to chitosan application concerning phenolic compounds accumulation in fruit tissues.

Chitosan treatment has been reported to have an influence on antioxidant enzyme activities in the tissues of both temperate and tropical fruit and vegetables (Tables 6-8). Compared to untreated strawberries, those treated with chitosan

maintained higher levels of antioxidant enzyme activities, such as catalase, glutathione-peroxidase, guaiacol peroxidase, dehydroascorbate reductase, and monodehydroascorbate reductase (Wang and Gao, 2012). Ascorbate peroxidase and glutathione reductase activities increased in pear treated with chitosan (Lin et al., 2008; Li et al., 2010a). Compared to the tissue of uncoated fruit, higher activities of superoxide dismutase, catalase, and peroxidase were reported after chitosan application to pear (Lin et al., 2008; Li et al., 2010a), sweet pepper (Xing et al., 2011a), and tropical fruit, such as guava (Hong et al., 2012). In addition, increased peroxidase activity after chitosan application has been reported for several other commodities, such as table grapes (Meng et al., 2008), pear (Meng et al., 2010a), sweet cherry (Dang et al., 2010), orange (Canale Rappussi et al., 2009), tomato (Liu et al., 2007), and potato (Xiao-Juan et al., 2008). Conversely, in other studies, decreased peroxidase activity was reported in litchi fruit after chitosan application, whether or not it was combined with other treatments (Zhang and Quantick, 1997; De Reuck et al., 2009; Sun et al., 2010). Meanwhile, treatment of litchi fruit with a combination of chitosan and ascorbic acid increased the activities of superoxide dismutase and catalase, and the contents of ascorbic acid and glutathione (Sun et al., 2010). Treatments with chitosan alone or in combination with *C. laurentii* decreased the superoxide dismutase activity in table grape tissues (Meng et al., 2008; 2010b; Meng and Tian, 2009). Treatments of navel oranges with 2% chitosan effectively enhanced the activities of peroxidase, superoxide dismutase and ascorbate peroxidase, but decreased the activities of catalase and the content of ascorbic acid (Zeng et al., 2010).

Physiological changes concerning polyphenol oxidase (PPO) activity have been observed after application of chitosan to fruit and vegetables (Tables 6-8). This has

great impact on fruit quality; indeed, PPO is a phenol-related metabolic enzyme that catalyzes the oxidation of phenolic compounds that are involved in plant defense against biotic and abiotic stresses and in pigmentation/ browning of fruit and vegetable tissues (Lattanzio et al., 2006; Bhattacharya et al., 2010). In some investigations, chitosan decreased PPO activity, and its inhibitory effects are probably a consequence of the adsorption of suspended PPO, its substrates, or its products by the positive charges of chitosan (Badawy and Rabea, 2009). The other possibility is that the selective permeability to gases due to the chitosan coating generates low levels of oxygen around the fruit surface, which can delay the deteriorative oxidation reactions, and partially inhibit the activities of oxidases such as PPO (Ayranci and Tunc, 2003). The chitosan coating markedly reduces PPO activity and delays skin browning during fruit shelf life. The maintenance of the skin color of the litchi fruit after chitosan treatment can be accounted for by the higher level of anthocyanin content in the skin that results from inhibition of PPO activity (Zhang and Quantick, 1997; Jiang et al., 2005; De Reuck et al., 2009). Similarly, the activities of PPO and peroxidase, and the related browning in the pericarp, were markedly lowered by treatment of harvested litchi fruits with ascorbic acid and 1% chitosan (Sun et al., 2010). In chitosan-treated tomato (Badawy and Rabea, 2009) and jujube (Wu et al., 2010; Xing et al., 2011b), the decreases in the PPO activities were concomitant with the enhanced phenolic content, and in sweet cherry (Dang et al., 2010), with the reduction in tissue browning. The combination of chitosan, calcium chloride and intermittent warming decreased the PPO activity in the tissues of peach that had been cold stored for 50 days (Ruoyi et al., 2005). However, in other investigations, PPO activities of fruit tissue increased after chitosan treatment. Chitosan treatment enhanced the activities of PPO in the flesh around the

wound of a pear (Meng et al., 2010a). An increase in the activity of PPO was demonstrated as a result of chitosan application in 'Valencia' oranges, which was seen 24 h after chitosan treatment (Canale Rappussi et al., 2009). Chitosan application in tomato fruit stored at 25 °C and 2 °C increased the content of the phenolic compounds and induced the activities of PPO, the levels of which were almost 1.5-fold those in the wounded control fruit at the same time (Liu et al., 2007). In this study, there was no direct relationship between the PPO activities and the content of phenolic compounds, although the phenolic compounds can be oxidized by the actions of PPO and peroxidase, to produce quinones (Campos-Vargas and Saltveit, 2002). It is likely that regulation of phenolic metabolism by the action of other enzymes, such as PAL, which participates in the biosynthesis of phenolic compounds, also has an important role (Liu et al., 2007). This could even explain the reason why in some investigations the PPO levels of fruit tissue after chitosan application are variable. Preharvest spraying with *C. laurentii* combined with postharvest chitosan coating increased the activities of PPO in table grapes during storage, but after 3 days of shelf life, the PPO activities in the treated fruit were lower than in the untreated fruit (Meng et al., 2010b). During cold storage, the PPO activity of litchi fruit coated with chitosan increased slowly, reached a peak, and then decreased (Zhang and Quantick, 1997).

EFFECT OF CHITOSAN TREATMENT ON MAINTENANCE OF FRUIT QUALITY AND RETENTION OF HEALTH-PROMOTING COMPOUNDS

Chitosan coating provides a semipermeable film around the fruit surface, which modifies the internal atmosphere by reducing oxygen and/or elevating carbon dioxide

levels, which decreases the fruit respiration level and metabolic activity, and hence delays the fruit ripening and senescence processes (Özden and Bayindirli, 2002; Olivas and Barbosa-Cánovas, 2005; Romanazzi et al., 2007; 2009; Vargas et al., 2008). A suppressed respiration rate slows down the synthesis and the use of metabolites, which results in lower soluble solids due to the slower hydrolysis of carbohydrates to sugars (Ali et al., 2011; Das et al., 2013). However, there are numerous confounding factors that can contribute to the soluble solids concentrations in fruit tissues; e.g., the fruit studied, its stage of ripeness, the storage conditions, and the thickness of the chitosan coating (Ali et al., 2011). On the other hand, as organic acids, such as malic and citric acid, are substrates for the enzymatic reactions of plant respiration, increased acidity and reduced pH would be expected in low-respiring fruit (Yaman and Bayindirli, 2001). Above all, the chitosan coating with its filmogenic properties has been used as a water barrier, to minimize water and weight loss of fruit during storage (Vargas et al., 2008; Bourlieu et al., 2009). All of these physiological changes have been reported in fruit and vegetables treated with chitosan (Tables 6-8).

For temperate fruit (Table 6), the chitosan coating minimized weight loss of stored apples, and its combination with heat treatment showed the lowest respiration rate, and significantly reduced pH and increased titratable acidity (Shao et al., 2012). Chitosan treatments of pears during storage reduced their vital activities, and in particular their respiration rate, which maintained the fruit quality and prolonged the shelf life. Compared with the control samples, chitosan-coated pears showed reduced weight loss (Zhou et al., 2008). Again in pear, chitosan coating alone and in combination with ascorbic acid resulted in decreased respiration rate, delayed weight loss, and retention of greater total soluble solids and titratable acidity (Lin et al., 2008).

Chitosan-treated peaches showed lower respiration rates and higher titratable acidity than control peaches (Li and Yu, 2001).

Chitosan forms a coating film on the outside surface of sweet cherries that effectively delayed the loss of water and promoted changes in titratable acidity and total soluble solids of the sweet cherries (Dang et al., 2010). Strawberries treated with chitosan alone or in combined with calcium gluconate showed reduced weight loss and respiration, which delayed the ripening and the progression of fruit decay due to senescence. Regardless of the addition of calcium gluconate to the chitosan, the coated strawberries had higher titratable acidity, and lower pH and soluble solids (Hernández-Muñoz et al., 2008). A chitosan coating without or with added calcium or vitamin E decreased weight loss and delayed the changes in pH and titratable acidity of strawberries and red raspberries during cold storage (Han et al., 2004; 2005). Chitosan application combined with bergamot oil provided a water vapor barrier for cold-stored table grapes, which reduced the fruit weight losses. Due to its hydrophobic nature, the addition of bergamot oil lowered this phenomenon further (Sánchez-González et al., 2011). Similarly, weight loss reductions in chitosan-coated table grapes were observed when this was combined with putrescine (Shiri et al., 2012) and grape seed extract (Xu et al., 2007b). The complex of zinc(II) and cerium(IV) with chitosan film-forming material that was applied to preserve the quality of Chinese jujube fruit reduced the fruit respiration rate and weight loss, while it increased the fruit total soluble solids, as compared to the uncoated fruit (Wu et al., 2010). In another study, after 42 days of storage at 13 °C, chitosan-coated citrus fruit showed less weight loss and higher titratable acidity and total soluble solids, compared to the control fruit. The weight loss of these citrus fruit decreased as the concentration of chitosan was increased (Chien and

712 Chou, 2006). Coating tomato fruit with chitosan solutions reduced the respiration rate
713 and ethylene production, with greater effects with 2% chitosan than 1% chitosan. The
714 chitosan coating increased the internal CO_2 and decreased the internal O_2 levels of the
715 tomatoes. These chitosan-coated tomatoes were also higher in titratable acidity (El
716 Ghaouth et al., 1992b).

717 Similar changes in respiration, weight loss, pH, titratable acidity, and soluble
718 solids content have been reported after chitosan treatment of tropical fruit (Table 7).
719 Polysaccharide-based coatings, including chitosan, applied to banana fruit reduced the
720 carbon dioxide evolution, loss of weight, and titratable acidity. Moreover, the reducing
721 sugar content and the total soluble solids of the coated fruit were lower than with the
722 untreated fruit, which suggests that the coated fruit synthesized reducing sugars at a
723 slower rate, through the slowed metabolism (Kittur et al., 2001). Similarly in bananas,
724 chitosan alone or in combination with 1-methylcyclopropene reduced the rate of
725 respiration (by 32%) compared to untreated banana, and decreased titratable acidity and
726 increased total soluble solids (Baez-Sañudo et al., 2009). The composite coating of
727 Arabic gum and chitosan provided an excellent semipermeable barrier around the
728 banana fruit, which reduced weight loss, modified the internal atmosphere, and
729 suppressed ethylene evolution, thus reducing respiration and delaying the ripening
730 process. After 33 days of storage, the soluble solids concentrations of the treated banana
731 fruit were lowered, whereas the titratable acidity was increased by the chitosan and
732 Arabic gum coating (Maqbool et al., 2010a; 2010b; 2011). The application of chitosan
733 delayed changes in eating quality, reduced respiration rate and weight loss, and
734 increased total soluble solid and titratable acidity of stored longan (Jiang and Li, 2001)
735 and guava (Hong et al., 2012) fruit. In mango fruit, the decline in respiration rate, fruit

weight, and titratable acidity were all effectively inhibited by chitosan (Jitareerat et al., 2007), while the increase in total soluble solids was delayed during storage (Zhu et al., 2008). Mango fruit coated with chitosan and subjected to hydrothermal treatment had less weight loss, lower pH and soluble solids, but higher acidity, regardless of the hydrothermal process (Salvador-Figueroa et al., 2011). The CO_2 concentration in the internal cavity of chitosan-treated papaya was significantly higher than that of the untreated fruit. The formation of a chitosan film on the fruit acted as a barrier for O_2 uptake, and slowed the rate of respiration and the metabolic activity, and consequently the ripening process (Hewajulige et al., 2009). Again in papaya, chitosan provided effective control of weight loss, and delayed the changes in soluble solids concentrations over 5 weeks of storage. The titratable acidity of the papaya fruit declined throughout the storage period, although at a slower rate in the chitosan-coated fruit, as compared to the untreated fruit (Bautista-Baños et al., 2003; Ali et al., 2010; 2011). Chitosan coating without or with calcium infiltration markedly slowed the ripening of papaya, as shown by their lack of weight loss, delay in titratable acidity decrease, and increase in soluble solids and pH (Al Eryani et al., 2008). In litchi fruit during storage, chitosan treatment produced an effective coating that reduced the respiration and transpiration of the fruit during storage (Lin et al., 2011), and reduced the decreases in the concentrations of total soluble solids and in the titratable acidity (Jiang et al., 2005). Similar results were obtained with the combination of chitosan with ascorbic acid, which significantly increased the titratable acidity and total soluble solids of stored litchi fruit (Sun et al., 2010).

Firmness is a major attribute that dictates the postharvest quality of fruit (Barrett et al., 2010). Fruit softening is a biochemical process that is normally attributed to the

deterioration of the cell-wall composition, which involves the hydrolysis of pectin by enzymes; e.g., polygalacturonase (Atkinson et al., 2012). Low levels of oxygen and higher levels of carbon dioxide restricts the activities of these enzymes and promotes the retention of fruit firmness during storage (Maqbool et al., 2011). Moreover, due to reduced transpiration, the water retention provides turgor to the fruit cells. Banana fruit treated with composite edible coatings of chitosan and Arabic gum showed significantly higher firmness than untreated bananas at the end of the storage period, and this firmness decreased as the concentration of the coating decreased (Maqbool et al., 2011). Chitosan coatings had beneficial effects on strawberry firmness, such that by the end of the storage period, the treated fruit had higher flesh firmness values than the untreated fruit (Hernández-Muñoz et al., 2008). In several other studies, chitosan coating maintained the firmness during storage of table grapes (Xu et al., 2007b; Sánchez-González et al., 2011), apple (Shao et al., 2012), pear (Lin et al., 2008), peach (Li and Yu, 2001), jujube (Qiuping and Wenshui, 2007), orange (Chien and Chou, 2006; Cháfer et al., 2012), banana (Kittur et al., 2001; Win et al., 2007; Baez-Sañudo et al., 2009), mango (Zhu et al., 2008; Salvador-Figueroa et al., 2011), papaya (Bautista-Baños et al., 2003; Sivakumar et al., 2005b; Ali et al., 2010; 2011), rambutan (Martínez-Castellanos et al., 2009), guava (Hong et al., 2012) and tomato (El Ghaouth et al., 1992b) (Tables 6-8).

In several studies, panelists were asked to observe and then rate the overall appearance, or just the flavor, of fruit treated or not with chitosan, using hedonic scales (Tables 6-8). These studies showed that chitosan can preserve the taste of pear fruit, which after cold storage was similar to the taste of the fresh fruit (Zhou et al., 2008). Similar results were obtained with the combination of chitosan and cinnamon oil

784 coating, which retained sweet pepper quality, without the development of off-flavors
785 (Xing et al., 2011a). Consumer acceptance based on color, flavor, texture, sweetness
786 and acidity was improved by chitosan coating and/or heat treatment of apple fruit (Shao
787 et al., 2012). For table grapes, chitosan alone and in combination with putrescine
788 prolonged the maintenance of the original sensory quality, in comparison with the
789 decline in the untreated grapes (Shiri et al., 2012). The combination of chitosan with
790 grape seed extract delayed rachis browning and dehydration, and maintained the visual
791 aspect of the berry without detrimental effects on taste or flavor (Xu et al., 2007b). In
792 sweet cherries, chitosan coating had a strong effect on the maintenance of quality
793 attributes, such as visual appearance, color, taste and flavor, as it had protective effects
794 in preventing surface browning, cracking, and the leaking of juice (Dang et al., 2010).
795 On strawberry, results from consumer sensory evaluations indicated that chitosan
796 increased the appearance and acceptance of the strawberries (Devlieghere et al., 2004),
797 whereas coatings containing chitosan and vitamin E developed a waxy-and-white
798 surface on the coated fruits (Han et al., 2005). In strawberries, the aroma and flavor of
799 chitosan-coated fruit was considered less intense than those of the uncoated fruit, which
800 were preferred by the panelists (Vargas et al., 2006). Likewise, panelists detected an
801 untypical oily aroma in samples coated with the combination of chitosan and oleic acid
802 (Vargas et al., 2006). On bananas, Baez-Sañudo et al. (2009) reported that chitosan
803 coating did not affect the sensory quality of the fruit. In another case, banana fruit
804 treated with 10% Arabic gum and 1% chitosan improved fruit quality during storage
805 and received the highest sensory scores for taste, pulp color, texture, flavor, and overall
806 acceptability (Maqbool et al., 2011). However, the fruit coated with high concentrations
807 of Arabic gum, as 15% or 20%, combined with 10% chitosan did not ripen fully after

about 1 month of storage, developed poor pulp color and inferior texture, and were off-flavored (Maqbool et al., 2011). Similarly, the sensory evaluation of papaya for taste, peel color, pulp color, texture, and flavor revealed that the fruit treated with 1.5% chitosan attained maximum scores from the panelists in all of the tested parameters. The untreated fruit and those treated with 0.5% chitosan ripened after 3 weeks of storage, and then began to decompose, while the fruit treated with 2% chitosan did not ripen fully after more than 1 month of cold storage. This was because of the thickness of the chitosan coating, which blocked the lenticels and caused fermentation inside, and in both cases the fruit were discarded from the evaluation due to the unacceptable quality. The flavor of the fruit with 1.5% chitosan coating was rated as excellent, because the pulp was not only sweet and pleasant, but also had a characteristic aroma (Ali et al., 2010; 2011). Litchi fruit subjected to chitosan treatment either alone or combined with carbonate salts showed good eating quality (Sivakumar et al., 2005a).

Several other investigations have reported changes after chitosan application to the color of the fruit peel, which were revealed either by technical instrumentation or by visual appearance (Tables 6-8). The application of chitosan coating in longan fruit delayed the fruit peel discoloration, which was related to the concomitant inhibition of PPO activity, the enzyme responsible for polyphenol oxidation (Jiang and Li, 2001). Papaya fruit treated with chitosan underwent light changes in peel color, as indicated by the slower increase in lightness and chroma values, as compared to uncoated fruit. The delay of color development for the papaya fruit treated with 1.0% 1.5% and 2.0% chitosan might be attributable to the slow rate of respiration and reduced ethylene production, which leads to delayed fruit ripening and senescence (Ali et al., 2011). Similarly, the combination of calcium and chitosan delayed surface color changes of

papaya fruit, as noted from the lower values of lightness and chroma and the higher value of hue angle in treated papaya, compared to untreated papaya (Al Eryani et al., 2008). During storage, chitosan coating delayed color changes in banana (Kittur et al., 2001; Win et al., 2007; Baez-Sañudo et al., 2009; Maqbool et al., 2011), litchi fruit (Zhang and Quantick, 1997; Caro and Joas, 2005; Joas et al., 2005; Ducamp-Collin et al., 2008; De Reuck et al., 2009; Sun et al., 2010), mango (Zhu et al., 2008; Salvador-Figueroa et al., 2011), citrus (Canale Rappussi et al., 2011), strawberry (Han et al., 2004; 2005; Hernández-Muñoz et al., 2008), and tomato (El Ghaouth et al., 1992b). Sensory analyses also revealed beneficial effects of chitosan coating in terms of delaying rachis browning and maintenance of the visual aspects of table grape berries (Xu et al., 2007b; Sánchez-González et al., 2011).

Fruit and vegetables treated with chitosan have a higher nutritional value, because chitosan can retain the contents of the ascorbic and phenolic compounds (Tables 6-8), which are positively correlated with antioxidant capacity (Rapisarda et al., 1999). Moreover, chitosan can be used as a vehicle for the incorporation of functional ingredients, such as other antimicrobials, minerals, antioxidants and vitamins. Some of these combinations can enhance the effects of chitosan or reinforce the nutritional value of the commodities (Vargas et al., 2008). Chitosan-based coatings can also carry high concentrations of calcium or vitamin E, thus significantly increasing the content of these nutrients in fresh and frozen strawberry and raspberry. Incorporation of calcium or vitamin E into chitosan-based coatings did not alter its antifungal properties, while it enhanced the nutritional value of these fresh and frozen strawberry and raspberry (Han et al., 2004). In addition, incorporation of calcium chloride in chitosan coating increased the stability of the cell wall and middle lamella of the strawberry tissue, and improved

its resistance to the pectic enzymes produced by fungal pathogens (Hernández-Muñoz et al., 2006; 2008). Calcium chloride has been added to chitosan coating for papaya (Al Eryani et al., 2008), pear (Yu et al., 2012) and peach (Ruoyi et al., 2005). Core browning is a major problem during storage in pear, and Lin et al. (2008) reported that the combination of chitosan with ascorbic acids not only controlled the core browning of pear, but also increased the ascorbic acid content and the antioxidant capacity of the pear. The combination of chitosan with ascorbic acid showed similar results as for pear (Lin et al., 2008) when applied to litchi fruit (Sun et al., 2010).

In the food industry, chitosan shows potential for application to food packaging, as a surrogate for petrochemical based films and as an innovative environmentally friendly material. This arises from its physico-chemical properties, its biodegradability, and its antifungal and antibacterial properties, with nontoxic and nonresidual effects (Porta et al., 2011; Schreiber et al., 2013). Considering the health conscious consumers and the carbon footprint on the environment, modern food packaging needs to address the application of bio-based active films or biopolymers, and chitosan shows potential as a bioagent or additive for the preparation of active films. Cervera et al. (2004) reported that chitosan films show higher oxygen barrier properties but lower water vapor barrier properties, mainly due to their hydrophilic nature. The water vapor permeability of chitosan films was shown to increase as a result of water interacting with the hydrophilic chitosan polymer. Incorporation of essential oils reduced the water vapor permeability and the films showed resistance to breaking and were less glossy and deformable; at the same time, the essential oils increased the antimicrobial properties of the coating (Zivanovic et al., 2005; Hosseini et al., 2008; Sánchez-González et al., 2011). Incorporating nanoparticles into the chitosan film (Qi et al.,

2004), such as ZnO (Li et al., 2010b) or Ag (Pinto et al., 2012) nanoparticles, improved the mechanical and barrier properties (Pereira de Abreu et al., 2007) and the thermal stability of the films (de Moura et al., 2009).

EFFECTS OF CHITOSAN ON FOODBORNE PATHOGENS

Foodborne illnesses are diseases that are caused by agents that enter the human body through the ingestion of food. In 2011, the Center for Disease Control and Prevention (CDC) estimated that in the United States each year there are 48 million foodborne illnesses that are responsible for 128,000 hospitalizations and 3,000 deaths (CDC, 2011). The World Health Organization (WHO) estimates that in 2005, 1.5 million people died worldwide from diarrheal diseases, with a great proportion of these cases being foodborne (WHO, 2006). Furthermore, in the future, with the growth of populations and movement of goods and people at the global scale, this might make the control of foodborne infections more difficult.

Recent investigations have identified fruit and vegetables, and in particular leafy greens, as important vehicles for the transmission of many foodborne disease outbreaks (Berger et al., 2010). Nowadays, there is increasing demand for fresh, minimally processed vegetables, such as ‘ready-to-eat’ salads, which retain much of their indigenous microflora following their minimal processing. All types of produce have the potential to harbor pathogens, and *Salmonell*a spp., *Shigella* spp., *Escherichia coli*, *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium* spp., *Aeromonas hydrophila*, some viruses, and other parasites are of the greatest public health interest (Beuchat, 2002). Fruit and vegetables can be

contaminated by these microorganisms during the preharvest stage, mainly by contaminated water or sewage and faeces, or during the postharvest stage, in the handling and storage of the horticultural products. The growth of microorganisms on fresh-cut produce can also occur during the cutting and slicing operations (Beuchat, 2002).

As well as its potentiality as a mechanical barrier, an edible chitosan coating can be used for its antimicrobial properties, to preserve fresh fruit and vegetables after harvest (Vargas et al., 2008). Some studies have reported on the antibacterial activities of chitosan films against foodborne pathogens of fresh fruit and vegetables (Table 9).

Inatsu et al. (2010) evaluated different sanitizers to prevent growth of four strains of *E. coli* on the surface of tomato fruit, and they found that 0.1% chitosan was effective when applied after a sodium chloride washing treatment. However, in this case, other combinations of sanitizers were more effective (e.g., 0.1% lactic acid with 0.05% sodium chloride). Chitosan coating reduced the native microflora on the surface of litchi fruit (Sivakumar et al., 2005a) and strawberry (Ribeiro et al., 2007), but not for table grapes (Romanazzi et al., 2002). However, several additives can be incorporated into the chitosan coating, which can provide more specific functions, such as antimicrobial activity that is aimed at either preventing or reducing the growth of foodborne microorganisms (Vargas et al., 2008). Coatings of chitosan and allyl isothiocyanate on cantaloupe reduced the *Salmonella* presence down to the limit of detection after 2 weeks of storage (Chen et al., 2012). Also when recontamination of cantaloupe with *Salmonella* was simulated, the results indicated that the chitosan-allyl isothiocyanate coating not only reduced the *Salmonella* more than the current practice based on acid washing, but it also maintained its antibacterial activity for longer periods

928 of time. Furthermore, the native microflora monitored by the microbial counts for total
929 aerobic bacteria, yeast and mold on the cantaloupe surface during storage were reduced
930 by the chitosan and allyl isothiocyanate coating (Chen et al., 2012). Essential oils are
931 among the antimicrobial agents that can be incorporated into chitosan coatings (Vargas
932 et al., 2008; Antunes and Cavaco, 2010). A coating of chitosan and bergamot oil
933 reduced the counts of molds, yeast, and mesophiles of table grape berries, as compared
934 to the untreated fruit. The addition of bergamot oil enhanced the antimicrobial activities
935 of the pure chitosan (Sánchez-González et al., 2011). In another study, growth of *E. coli*
936 DH5α did not take place when the bacterium was incubated on substrates with added
937 chitosan and beeswax, without or with added thyme or lime essential oils (Ramos-
938 García et al., 2012).

939 The antimicrobial activity of chitosan appears to be due to its polycationic
940 characteristics, which allow chitosan to interact with the electronegative charges on the
941 cell surface of fungi and bacteria. This can result in increased microbial cell
942 permeability, internal osmotic disequilibrium, and cell leakage (Helander et al., 2001;
943 Rabea et al., 2003; Liu et al., 2004; Raafat et al., 2008; Mellegård et al., 2011). A 12-h
944 exposure period to chitosan resulted in higher levels of glucose and protein in the
945 supernatant of cell suspensions of *Staphylococcus aureus* than observed for the medium
946 without chitosan. The reactive amino groups in chitosan might conceivably interact with
947 a multitude of anionic groups on the cell surface, to alter cell permeability and cause
948 leakage of intracellular components, such as glucose and protein, which will lead to cell
949 death (Chung et al., 2011). Furthermore, the possibility of a direct interaction of
950 chitosan with negatively charged nucleic acids of microorganisms, and consequently of
951 chitosan interference in RNA and protein synthesis, has been proposed (Rabea et al.,

2003). In contrast, Raafat et al. (2008) considered the probabilities of the penetration of chitosan into the nuclei of bacteria to be relatively low, as the size of a molecule of hydrated chitosan is bigger than the cell wall pores. Thus Raafat et al. (2008) examined cell damage of *Staphylococcus simulans* after exposure to chitosan, and they found irregular structures that protruded from the cell wall and a 'vacuole-like' structure that possibly resulted from disruption of the equilibrium of the cell-wall dynamics, such as the ion and water efflux, and decreased the internal pressure; however, on the other hand, the cell membrane remained intact. These results show how chitosan appears not to interact directly with internal structures of the bacteria, but to just interact with external cell-wall polymers. Other mechanisms proposed for the chitosan antimicrobial activity are based on the strong affinity of chitosan for nutritionally essential metal ions. Rabea et al. (2003) reported that the binding of bacterial trace metals by chitosan inhibited both microbial growth and the production of bacterial toxins.

The susceptibility of foodborne microorganisms to chitosan also depends on the characteristics of the microorganisms themselves. As the antimicrobial activity of chitosan relies on electrostatic interactions, the nature of the bacterial cell wall can influence the inhibition of microorganism growth by chitosan. The main important foodborne microorganisms are Gram-negative and Gram-positive bacteria. *E. coli*, *Salmonella* spp., *Shigella* spp., *A. hydrophila*, *C. jejuni* and *Y. enterocolitica*, are Gram-negative, and they are characterized by an outer cell wall that consists essentially of lipopolysaccharides that contain phosphate and pyrophosphate groups that cover their surface with negative charges. The gram-positive bacteria, such as *L. monocytogenes*, *B. cereus*, and *C. botulinum*, have a cell wall that is composed essentially of peptidoglycan associated to polysaccharides and teichoic acids, which are also negatively charged.

According to several studies, Gram-positive bacteria are more susceptible to chitosan than Gram-negative bacteria (No et al., 2002; Takahashi et al., 2008; Jung et al., 2010; Tayel et al., 2010), while according to others, the opposite is the case (Devlieghere et al., 2004). A recent study reported the effectiveness of chitosan and its derivatives against well-established biofilms formed by foodborne bacteria, which are assumed to be resistant to cleaning and disinfection practices. The results showed that a 1 h exposure to chitosan resulted in reductions in viable cells on mature *L. monocytogenes* biofilms, and in the attached populations of the other organisms tested, as *B. cereus*, *Salmonella enterica* and *Pseudomonas fluorescens*, except for *S. aureus* (Orgaz et al., 2011).

In the food industry, chitosan is frequently used as an antioxidant, a clarifying agent, and an inhibitor of enzymatic browning. When applied to food, the antimicrobial activities of chitosan can be affected by the pH or the matrix. Indeed, the pKa of chitosan, where half of its amino group are protonated and half are not, is around 6.5; therefore, this means that at pH <6.5, the protonated form of chitosan predominates, which results in a greater positive charge density, and leads to stronger and more frequent electrostatic interactions, and thus to greater antimicrobial effectiveness (Helander et al., 2001; Devlieghere et al., 2004; Jung et al., 2010; Kong et al., 2010). This was illustrated by the growth of *Candida lambica*, which was completely inhibited at pH 4.0, while at pH 6.0, the same chitosan concentration led to a relatively small decrease in growth rate (Devlieghere et al., 2004). Furthermore, this also explains why chitosan is less soluble in water alone than in solutions with acids, where chitosan shows more positive charges, and therefore a greater number of interactions. Chitosan with a higher degree of deacetylation, which has greater numbers of positive charges,

would also be expected to have stronger antibacterial activities (Jung et al., 2010; Kong et al., 2010; Tayel et al., 2010). On the other hand, numerous studies have generated different results relating to correlations between the chitosan bactericidal activities and its molecular weight. In some studies, lower molecular weight chitosans (ranging from 2.7 $\times 10^4$ to 5.5 $\times 10^4$ Da) was more effective against bacteria than higher molecular weight chitosans (Liu et al., 2006; Tayel et al., 2010; Kim et al., 2011). In other studies, this trend was observed against Gram-negative bacteria, but not against Gram-positive bacteria (No et al., 2002; Zheng and Zhu, 2003). According to Benhabiles et al. (2012), when the molecular weight of chitosan is low, its polymer chains have greater flexibility to create more bonds, and they can thus better interact with the microbial cells. In other studies, no trends were reported for the antibacterial actions related to increased or decreased molecular weights of chitosan (Jung et al., 2010; Mellegård et al., 2011).

CONCLUSIONS AND FUTURE TRENDS

This review reports on the recent and most relevant studies concerning preharvest spraying and postharvest application of chitosan for fruit and vegetables. These studies have shown that this biopolymer can effectively maintain the fruit and vegetable quality, and can control their postharvest decay during storage and shelf life. Studies dealing with the mechanisms of action of chitosan as an antimicrobial against postharvest fungi and foodborne bacteria are also summarized here. The film-forming properties, antimicrobial activities, and induction of plant resistance of chitosan appear to be the main factors in its success. With its intrinsic properties, and because of its double activity on the host and the pathogen, chitosan can be considered as the first of a

new class of plant-protection products (Bautista-Baños et al., 2006). Moreover, chitosan has been under considerable investigation for applications in biomedicine, pharmacology, biotechnology, and in the food industry, due to its biocompatibility, biodegradability, and bioactivity (Synowiecki and Al-Khateeb, 2003; Tharanathan and Kittur, 2003; Wu et al., 2005). Chitosan is not toxic to humans and its safe use as a pharmaceutical carrier has been reported (Baldrick, 2010; USFDA, 2013).

Chitosan has been reported to be a potentially viable alternative for fruit and vegetable preservation. Multicomponent edible coatings can be produced with suitable ingredients for the product to provide the desired barrier protection, while also serving as a vehicle for the incorporation of specific additives that can enhance the functionality, such as antioxidants and antimicrobials, thus avoiding pathogen or foodborne microorganism growth on the surface of fruit and vegetable products (Valencia-Chamorro et al., 2011). The combination of chitosan with minerals, vitamins or other nutraceutical compounds can reinforce the nutritional value of the commodities, without reducing the taste acceptability. This new generation of edible coatings is being especially designed to allow incorporation and/or controlled release of antioxidants, vitamins, nutraceuticals, and natural antimicrobial agents (Vargas et al., 2008; McClements et al., 2009).

The availability of commercial chitosan products that are easily dissolvable in water now provides an alternative to synthetic fungicides for growers, for the control of diseases of fruit and vegetables. However, at present, none of the formulations of chitosan are registered as plant protectant products. The present review summarizes the application of chitosan either preharvest or postharvest. Here, postharvest treatment is not advisable for fruit that are characterized by a bloom on the surface, such as table

grapes, or that have a thin waxy pericarp and succulent flesh, such as strawberries, which can be easily damaged during harvest and postharvest handling. On these commodities, preharvest treatment (even 1-2 days before harvest) can be considered as a promising approach to control the postharvest decay of these fruit under storage. Although a lot of information regarding the effectiveness of chitosan in the control of postharvest decay of fruit and vegetables is available, its application to large-scale tests and its integration into commercial agricultural practices are key points that need to be investigated further. In addition, more studies concerning the exact mechanisms of action of chitosan are needed. Also, several mechanisms relating to its antifungal and antibacterial activities remain unclear. New knowledge about these aspects will provide the necessary information to support decisions relating to the preparation of the chitosan, which molecular weight chitosan to use, and the kind of commercial formulation.

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1699 **Table 1**. Chitosan-based commercial products that are available for the control of postharvest diseases.

Product trade name	Company (Country)	Formulation	a.i. (%)	Fruit/vegetable	Reference
Chito Plant	ChiPro GmbH (Bremen, Germany)	Powder	99.9	Table grapes, sweet cherry, strawberry	Feliziani et al., 2013a; 2013b; Romanazzi et al., 2013
OIIYS	Venture Innovations (Lafayette, LA, USA)	Liquid	5.8	Table grapes	Feliziani et al., 2013a
Armour-Zen	Botry-Zen Limited (Dunedin, New Zealand)	Liquid	14.4	Peach, table grapes	Casals et al., 2012; Calvo-Garrido et al., 2013; Feliziani et al., 2013a
Biorend	Bioagro S.A. (Chile)	Liquid	1.25	Clementine, mandarin fruit	Fornes et al., 2005
FreshSeal	BASF Corporation (Mount Olive, NJ, USA)	Liquid	n.d.	Banana	Baez-Sañudo et al., 2009
ChitoClear	Primex ehf (Siglufjordur, Iceland)	Powder	100	Rambutan fruit	Martínez-Castellanos et al., 2009
Bioshield	Seafresh (Bangkok, Thailand)	Powder	100	Mango	Jitarrerat et al., 2007
Biochikol 020 PC	Gumitex (Lowics, Poland)	Liquid	2	Potato	Kurzawińska and Mazur, 2007

1700 a.i., active ingredient

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1702 **Table 2**. Chitosan treatments with other applications for storage decay of temperate fruit.

Fruit	Decay	Integration to chitosan	References (application time)
Table grape	Gray mold	-	Romanazzi et al., 2002 (pre- and postharvest)
		Acid solutions	Romanazzi et al., 2009 (postharvest)
		Ethanol	Romanazzi et al., 2007 (postharvest)
		Grape seed extract	Xu et al., 2007b (postharvest)
	Gray mold and blue mold	UV	Romanazzi et al., 2006 (preharvest)
	Decay in general	*Cryptococcus laurentii*	Meng and Tian, 2009 (preharvest); 2010a (postharvest)
Strawberry	Gray mold	-	El Ghaouth et al., 1991a; 1992a (postharvest); Zhang and Quantick, 1998 (postharvest); Romanazzi et al., 2000 (pre and postharvest); Reddy et al., 2000a (preharvest); Mazaro et al., 2008 (preharvest)
		Lemon essential oil	Perdones et al., 2012 (postharvest)
		Red thyme, oregano extract, limonene, peppermint	Vu et al., 2011(postharvest)
	Rhizopus rot	-	El Ghaouth et al., 1992a (postharvest); Zhang and Quantick, 1998 (postharvest); Romanazzi et al., 2000 (pre and postharvest); Park et al., 2005 (postharvest)
	Cladosporium rot	-	Park et al., 2005 (postharvest)
	Decay in general	Calcium lactate + calcium gluconate, vitamin E	Han et al., 2004 (postharvest)
		Calcium gluconate	Hernández-Muñoz et al., 2006 (postharvest); 2008 (postharvest)
		Oleic acid	Vargas et al., 2006 (postharvest)
Raspberry	Decay in general	Calcium lactate, calcium gluconate, vitamin E	Han et al., 2004 (postharvest)
	Gray mold and Rhizopus rot	-	Zhang and Quantick, 1998 (postharvest)

Fruit	Decay	Integration to chitosan	References (application time)
Blueberry	Decay in general	-	Duan et al., 2011 (postharvest)
Apple	Blue mold	UV-C, *Candida satoiana*, harpin	De Capdeville et al., 2002 (postharvest)
		Cryptococcus laurentii	Yu et al., 2007 (postharvest)
		Candida satoiana	El Ghaout et al., 2000 (postharvest)
		Heat treatment	Shao et al., 2012 (postharvest)
	Gray mold	*Candida satoiana*	El Ghaout et al., 2000 (postharvest)
		Heat treatment	Shao et al., 2012 (postharvest)
Pear	Blue mold	Calcium chloride + *Cryptococcus laurentii*	Yu et al., 2012 (postharvest)
Peach	Brown rot	-	Li and Yu, 2001 (postharvest)
		Heat treatment	Casals et al., 2012 (postharvest)
Sweet cherry	Decay in general	Hypobaric treatment	Romanazzi et al., 2003 (pre- and postharvest)
		-	Romanazzi et al., 1999 (preharvest); Feliziani et al., 2013b (pre- and postharvest)
Orange	Blue mold	Bergamot, thyme, tea tree essential oil	Cháfer et al., 2012 (postharvest)
	Black spot disease	-	Canale Rappussi et al., 2009; 2011 (postharvest)
Tankan citrus fruit	Decay in general	-	Chien and Chou, 2006 (postharvest)
Clementine mandarin fruit	Decay in general	-	Fornes et al., 2005 (pre- or postharvest)

1705 **Table 3**. Chitosan treatments with other applications for storage decay of tropical fruit.

Fruit	Decay	Integration to chitosan	References (application time)
Banana	Anthracnose	-	Zahid et al., 2012 (postharvest)
		Arabic gum	Maqbool et al., 2010a; 2010b (postharvest)
	Crown rot	Cinnamon extract	Win et al., 2007 (postharvest)
Mango	Anthracnose	-	Zhu et al., 2008 (postharvest); Abd-Alla and Haggag, 2010 (postharvest)
		Irradiation	Abbasi et al., 2009 (postharvest)
Papaya	Anthracnose	-	Hewajulige et al., 2009 (postharvest); Ali et al., 2010 (postharvest); Zahid et al., 2012 (postharvest)
		Aqueous extract of papaya seeds	Bautista-Baños et al., 2003 (postharvest)
		Ammonium carbonate, sodium bicarbonate	Sivakumar et al., 2005b (postharvest)
Dragon fruit	Anthracnose	-	Zahid et al., 2012 (postharvest)
Litchi fruit	Blue mold and Cladosporium rot	Potassium metabisulfite	Sivakumar et al., 2005a (postharvest)
Longan fruit	Decay in general		Jiang and Li, 2001 (postharvest)

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1708 **Table 4**. Chitosan treatments with other applications for storage decay of vegetables.

Vegetable	Decay	Integration to chitosan	References (application time)
Tomato	Gray mold		El Ghaouth et al., 1992b (postharvest); Badawy and Rabea, 2009 (postharvest)
	Gray mold and blue mold		Liu et al., 2007 (postharvest)
	Blackmold Rot		Reddy et al., 2000b (postharvest)
Sweet pepper	Decay in general	Cinnamon oil	Xing et al., 2011a (postharvest)
Melon	Fusarium rot and alternaria rot	Natamycin	Cong et al., 2007 (postharvest)

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1711 **Table 5**. Growth inhibition of chitosan on decay-causing fungi that affect the produce during storage.

Fungus	Infected species	Reference
Alternaria alternata	Tomato	Sánchez-Domínguez et al., 2011
Alternaria kikuchiana	Pear	Meng et al., 2010a
Aspergillus phoenicus	Pear	Cè et al., 2012
Aspergillus niger		Plascencia-Jatomea et al., 2003
Botrydiplodia lecanidion	Tankan citrus fruit	Chien and Chou, 2006
Botrytis cinerea	Tomato, potato, bell pepper fruit, cucumber, peach, strawberries, table grapes, pear, apple, Tankan citrus fruit	El Ghaouth et al., 1992a; 2000; Du et al., 1997; Romanazzi et al., 2002; Ben-Shalom et al., 2003; Ait Barka et al., 2004; Badawy et al., 2004; Chien and Chou, 2006; Lira-Saldivar et al., 2006; Elmer and Reglinski, 2006; Liu et al., 2007; Xu et al., 2007b; Badawy and Rabea, 2009; Rabea and Badawy, 2012
Cladosporium spp.	Litchi fruit, strawberry	Park et al., 2005; Sivakumar et al., 2005a
Colletotrichum gloeosporioides	Mango, papaya	Bautista Baños et al., 2003; Sivakumar et al., 2005b; Jitareerat et al., 2007; Ali and Mahmud, 2008; Hewajulige et al., 2009; Abd-Alla and Haggar, 2010; Ali et al, 2010; Zahid et al., 2012
Colletotrichum musae	Banana	Win et al., 2007; Maqbool et al., 2010a; 2010b; Zahid et al., 2012
Colletotrichum spp.	Table grapes and tomato	Muñoz et al., 2009
Fusarium solani		Eweis et al., 2006
Fusarium sulphureum	Potato	Yong-Cai, et al., 2009
Fusarium spp.	Banana	Win et al., 2007
Geotricum candidum		El-Mougy et al., 2012
Guignardia citricarpa	Orange	Canale Rappussi et al., 2009; 2011
Lasiodiplodia theobromae	Banana	Win et al., 2007
Monilinia fructicola	Apple, peach	Yang et al., 2010; 2012
Monilinia laxa	Sweet cherry	Feliziani et al., 2013b
Penicillium citrinum	Jujube	Xing et al., 2011b
Penicillium digitatum	Orange, lemon, Tankan citrus fruit	El Ghaouth et al., 2000; Bautista Baños et al., 2004; Chien and Chou, 2006; El-Mougy et al., 2012
Penicillium expansum	Litchi fruit, strawberries, apple,	El Ghaouth et al., 2000; Sivakumar et al., 2005a; Liu et al., 2007;

Fungus	Infected species	Reference
	pear, tomato	Yu et al., 2007
Penicillium italicum	Tankan citrus fruit	Chien and Chou, 2006; El-Mougy et al., 2012
Penicillium stolonifer	Pear	Cè et al., 2012
Phytophthora cactorum	Strawberries	Eikemo et al., 2003
Physalospora piricola	Pear	Meng et al., 2010a
Rhizopus stolonifer	Peach, strawberries, papaya, tomato	El Ghaouth et al., 1992a; Bautista Baños et al., 2004; Park et al., 2005; Guerra-Sánchez et al., 2009; García-Rincón et al., 2010; Hernández-Lauzardo et al., 2010; Ramos-García et al., 2012
Sclerotinia sclerotiorum	Carrot	Cheah et al., 1997; Molloy et al., 2004; Ojaghian et al., 2013

1714 **Table 6**. Physiological changes that can occur in temperate fruit after chitosan treatment.

Fruit	Physiological change	Integration to chitosan	References
Table grapes	Phenylalanine ammonia-lyase	-	Romanazzi et al., 2002; Meng et al., 2008
		Cryptococcus laurentii	Meng and Tian, 2009; Meng et al., 2010b
	Peroxidase	-	Meng et al., 2008; Gao et al., 2013
	Polyphenol oxidase, superoxide dismutase	-	Meng et al., 2008; Gao et al., 2013
		Cryptococcus laurentii	Meng and Tian, 2009; Meng et al., 2010b
	Chitinase, myricetin	-	Feliziani et al., 2013a
	Quercetin	-	Feliziani et al., 2013a
		Putrescine	Shiri et al., 2012
	Respiration	Bergamot oil	Sánchez-González et al., 2011
	Trans-resveratrol	UV	Romanazzi et al., 2006
		-	Feliziani et al., 2013a
	Soluble solids content	-	Meng et al., 2008
		Bergamot oil	Sánchez-González et al., 2011
		Cryptococcus laurentii	Meng et al., 2010b
		Glucose	Gao et al., 2013
	Titratrable acidity	-	Meng et al., 2008
	Total phenolic content	-	Meng et al., 2008
		Cryptococcus laurentii	Meng et al., 2010b
		Putrescine	Shiri et al., 2011
	Weight loss, color, texture	Bergamot oil	Sánchez-González et al., 2011
		Putrescine	Shiri et al., 2012
		Grape seed extract	Xu et al., 2007b
		Glucose	Gao et al., 2013
	Shattering and cracking	Putrescine	Shiri et al., 2012
		Grape seed extract	Xu et al., 2007b
Strawberries	Titratable acidity	-	El Ghaouth et al., 1991a; Zhang and Quantick, 1998; Reddy et al., 2000a
		Vitamin E	Han et al., 2004; 2005

Fruit	Physiological change	Integration to chitosan	References
		Calcium gluconate	Hernández- Muñoz et al., 2008
	pH	Calcium gluconate	Hernández-Muñoz et al., 2008
		Vitamin E	Han et al., 2004
	Antocyanin content	-	El Ghaouth et al., 1991a; Zhang and Quantick, 1998; Reddy et al., 2000a
		Oleic acid	Vargas et al., 2006
	Total polyphenol	-	Kerch et al., 2011
	Soluble solids content	Vitamin E	Han et al., 2005
	Colour	Calcium gluconate	Hernández-Muñoz et al., 2008
		Vitamin E	Han et al., 2004; 2005
	Firmness	Calcium gluconate	Hernández-Muñoz et al., 2008
		-	El Ghaouth et al., 1991a
	Vitamin C content	-	Zhang and Quantick, 1998; Kerch et al., 2011; Wang and Gao, 2012
	Glutathion	-	Wang and Gao, 2012
	Chitinase	-	Zhang and Quantick, 1998; Landi et al., 2014
	β-1,3 glucanase	-	Zhang and Quantick, 1998; Landi et al., 2014
	Phenilalanine ammonia-lyase	-	Romanazzi et al., 2000; Landi et al., 2014
	Weight loss	Vitamin E	Han et al., 2004
	Respiration	-	El Ghaouth et al., 1991a; Vargas et al., 2006
	Chalcone isomerase, flavonol synthase, anthocyanidin synthase, calcium-dependent protein kinase, potassium channel, PR-1, polygalacturonase, polygalacturonase inhibiting protein	-	Landi et al., 2014
	Catalase, glutathione-peroxidase, guaiacol peroxidase, dehydroascorbate reductase, monodehydroascorbate	-	Wang and Gao, 2012

Fruit	Physiological change	Integration to chitosan	References
	reductase		
Raspberry	Weigth loss, color, pH, titratable acidity	Vitamin E	Han et al., 2004
	Ascorbic acid, titratable acidity, firmness, antocyanin content	-	Zhang and Quantick, 1998
Apple	Respiration, firmness, weicht loss, titratable acidity	Heat	Shao et al., 2012
Pear	Polyphenol oxidase, chitinase, β-1,3 glucanase,	-	Meng et al., 2010b
	ROS, catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase		Li et al., 2010a
	Peroxidase	-	Meng et al., 2010b; Li et al., 2010a
	Respiration, permeability of cell membrane, weight loss	-	Zhou et al., 2008
		Ascorbic acid	Lin et al., 2008
	Soluble solid contents, titratable acidity, firmness	Ascorbic acid	Lin et al., 2008
Apricot	Total phenolic content, antioxidant activity, weight loss	-	Ghasemnezhad et al., 2010
Peach	Titratable acidity, ascorbic acid, respiration, firmness, ethylene and malondialdehyde production, superoxide dismutase	-	Li and Yu, 2001
	Polyphenol oxidase, peroxidase, ascorbic acid oxidase, polygalacturonase, vitamic C	$CaCl_2$ coating + PEpackage + intermittent warming	Ruoyi et al., 2005
Sweet cherry	Titratable acidity, soluble solid, catalase, peroxidase, polyphenol oxidase, phenilalanine ammonia-lyase, respiration	-	Dang et al., 2010
	Ascorbic acid	-	Dang et al., 2010; Kerch et al., 2011
	Phenols content, antocyanin content	-	Kerch et al., 2011
Orange	Water loss, firmness	Bergamot, thyme, tea tree essential oil	Cháfer et al., 2012
	Color	-	Canale Rappussi et al., 2011
	Chitinase, b-1,3-glucanase, polyphenol oxidase	-	Canale Rappussi et al., 2009
	Peroxidase	-	Canale Rappussi et al., 2009; Zeng et al.,

Fruit	Physiological change	Integration to chitosan	References
			2010
	Superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, hydrogen peroxide content, ascorbate content	-	Zeng et al., 2010
Tankan citrus fruit	Firmness, weight loss, titratable acidity, ascorbic acid, soluble solids	-	Chien and Chou, 2006
Jujube	Polyphenol oxidase, phenolic compounds	-	Xing et al., 2011b
		Zinc, cerium	Wu et al., 2010
	Ascorbic acid	-	Xing et al., 2011b
		1-methylcyclopropene	Qiuping and Wenshui, 2007
	Firmness	1-methylcyclopropene	Qiuping and Wenshui, 2007
	Weight loss	1-methylcyclopropene	Qiuping and Wenshui, 2007
		Zinc, cerium	Wu et al., 2010
	Respiration, soluble solids	Zinc, cerium	Wu et al., 2010

1717 **Table 7**. Physiological changes that can occur in tropical fruit after chitosan treatment.

Fruit	Physiological changes	Integration to chitosan	References
Banana	Titratable acidity	-	Kittur et al., 2001
		1-methylcyclopropene	Baez-Sañudo et al., 2009
		Arabic gum	Maqbool et al., 2010a, 2010b
	Respiration	-	Kittur et al., 2001
		1-methylcyclopropene	Baez-Sañudo et al., 2009
		Arabic gum	Maqbool et al., 2011
	Firmness, soluble solids content	-	Kittur et al., 2001; Win et al., 2007
		1-methylcyclopropene	Baez-Sañudo et al., 2009
		Arabic gum	Maqbool et al., 2010a; 2010b; 2011
	Color change	-	Kittur et al., 2001; Win et al., 2007
		1-methylcyclopropene	Baez-Sañudo et al., 2009
		Arabic gum	Maqbool et al., 2011
	Weight loss	Arabic gum	Maqbool et al., 2010a; 2010b; 2011
Longan fruit	Respiration, weight loss, color change, polyphenol oxidase, titratable acidity, total soluble solids, ascorbic acid	-	Jiang and Li, 2001
Mango	Titratable acidity, weight loss	-	Jitareerat et al., 2007; Zhu et al., 2008
		Hydrothermal process	Salvador-Figueroa et al., 2011
	Total soluble solids, firmness, color change	-	Zhu et al., 2008
		Hydrothermal process	Salvador-Figueroa et al., 2011
	pH	Hydrothermal process	Salvador-Figueroa et al., 2011
	Chitinase, b-1,3-glucanase	-	Jitareerat et al., 2007
	Respiration, ascorbic acid	-	Jitareerat et al., 2007; Zhu et al., 2008
Papaya	Titratable acidity, total soluble solids	-	Ali et al., 2010; 2011
		Calcium infiltration	Al Eryani et al., 2008
	Ascorbic acid	-	Ali et al., 2011

Fruit	Physiological changes	Integration to chitosan	References
		Calcium infiltration	Al Eryani et al., 2008
	Weight loss, color change	-	Ali et al., 2011
		Ammonium carbonate, sodium bicarbonate	Sivakumar et al., 2005b
		Calcium infiltration	Al Eryani et al., 2008
	Firmness	-	Ali et al., 2010; 2011
		Ammonium carbonate, sodium bicarbonate	Sivakumar et al., 2005b
		Aqueous extract of papaya seeds	Bautista-Baños et al., 2003
	Chitinase, b-1,3-glucanase	-	Hewajulige et al., 2009
	Respiration		Hewajulige et al., 2009, Ali et al., 2011
Litchi fruit	Weight loss	-	Zhang and Quantick, 1997; Jiang and Li, 2001; Sivakumar et al., 2005a; Sun et al., 2010; Lin et al., 2011
		Organic acids	Joas et al., 2005; Caro and Joas, 2005
	Titratable acidity	-	Jiang et al., 2005; Sivakumar et al., 2005a; Sun et al., 2010
		Organic acids	Joas et al., 2005; Caro and Joas, 2005
	Total phenolic content, flavonoid content	-	Zhang and Quantick, 1997; Sivakumar et al., 2005a
	Anthocyanin content	-	Zhang and Quantick, 1997; Jiang et al., 2005; Sivakumar et al., 2005a;
		Modified atmosphere packaging	De Reuck et al., 2009
	Respiration rate	-	Lin et al., 2011
	Color change	-	Zhang and Quantick, 1997; Ducamp-Collin et al., 2008
		Organic acids	Caro and Joas, 2005; Joas et al., 2005
		Ascorbic acid	Sun et al., 2010
		Modified atmosphere packaging	De Reuck et al., 2009

Fruit	Physiological changes	Integration to chitosan	References
	Total soluble solid	-	Jiang et al., 2005
		Ascorbic acid	Sun et al., 2010
	Peroxidase	-	Zhang and Quantick, 1997; Dong et al., 2004
		Ascorbic acid	Sun et al., 2010
		Modified atmosphere packaging	De Reuck et al., 2009
	Polyphenol oxidase	-	Zhang and Quantick, 1997; Jiang et al., 2005; Lin et al., 2011
		Ascorbic acid	Sun et al., 2010
		Modified atmosphere packaging	De Reuck et al., 2009
	Super oxide dismutase, catalase, hydrogen peroxide, malondialdeyde; ascorbic acid content	Ascorbic acid	Sun et al., 2010
Rambutan	Firmness, soluble solid, titratable acidity	*Lactobacillus plantatum*	Martínez-Castellanos et al., 2009
Guava	Firmness, peroxidase superoxide dismutase, catalase, inhibition of superoxide free radical production, titratable acidity, ascorbic acid, weight loss, soluble solids, chlorophyll and malondialdehyde content	-	Hong et al., 2012

1720 **Table 8**. Physiological changes that can occur in vegetables after chitosan treatment.

Vegetables	Physiological changes	Integration to chitosan	References
Tomato	Respiration rate, color change, ethylene production, firmness, titratable acidity	-	El Ghaouth et al., 1992b
	Polyphenol oxidase, phenolic content	-	Liu et al., 2007; Badawy and Rabea, 2009
	Peroxidase	-	Liu et al., 2007
	Protein content	-	Badawy and Rabea, 2009
	Polygalacturonase, pectate lyase, cellulose, phytoalexin production, pH	-	Reddy et al., 2000b
Potato	Peroxidase, polyphenol oxidase, flavonoid content, lignin content	-	Xiao-Juan et al., 2008
	Phenylalanine ammonia-lyase	-	Gerasimova et al., 2005
Sweet pepper	Superoxide dismutase, peroxidase, catalase	Cinnamon oil	Xing et al., 2011a
	Respiration, weight loss, color	-	El Ghaouth et al., 1991b
Cucumber	Respiration, weight loss, color	-	El Ghaouth et al., 1991b
Melon	Weigth loss, ascorbic acid, pH	Natamycin	Cong et al., 2007
Carrot	Polyphenol oxidase, peroxidase, phenylalanine ammonia-layse	-	Ojaghian et al., 2013

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1723 **Table 9**. Application of chitosan on fruit and vegetable to control foodborne microorganisms.

Microorganism	Substrate of growth	Integration to chitosan	References
Escherichia coli	Tomato	-	Inatsu et al., 2010
	Tomato	Beeswax + lime essential oil	Ramos-García et al., 2012
Salmonella spp.	Whole cantaloupe	Allyl isothiocyanate, nisin	Chen et al., 2012

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