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Shelf life extension of fresh fruit and vegetables by chitosan treatment

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(Article begins on next page)

1 **Shelf Life Extension of Fresh Fruit and Vegetables by Chitosan**
2 **Treatment**

3

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

39

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

42

43

44 *INTRODUCTION*

45

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

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

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

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

82 Nowadays, commercial chitosan formulations are available on the market. Some
83 commercial formulations have been tested for the control of postharvest diseases in
84 different fresh produce commodities, as shown in Table 1. The commercial
85 formulations used in plant disease management, not only for the control of postharvest
86 decay of fruit, include: Chitogel (Ecobulle, France) (Ait Barka et al., 2004; Elmer and
87 Reglinski, 2006); Biochikol 020 PC (Gumitex, Lowics, Poland) (Nawrocki, 2006);
88 Armour-Zen (Botry-Zen Limited, Dunedin, New Zealand) (Reglinski et al., 2010);
89 Elexa 4 Plant Defense Booster (Plant Defense Booster Inc., USA) (Elmer and Reglinski,
90 2006); and Kendal Cops (Iriti et al., 2011). The main differences between practical

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

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

105

106 *INFLUENCE OF PREHARVEST CHITOSAN APPLICATION OF ON POSTHARVEST*
107 *DISEASE CONTROL*

108

109 Although many studies have reported on the effectiveness of chitosan treatments at the
110 postharvest stage, the research findings on the evaluation of the preharvest application
111 of chitosan on the control of postharvest decay in fresh produce is limited (Tables 2-4).
112 However, chitosan applications prior to harvest might be suitable for fruit, such as table
113 grapes and strawberries, because these fruit have a bloom on the surface and/or can
114 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 SO₂ 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/cm² 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

139 among the three that were tested. Other rot diseases that were mainly caused by
140 *Alternaria* spp. and *Penicillium* spp. were mainly reduced by the OII-YS chitosan
141 formulation, which was even more effective than the fungicide program (Feliziani et al.,
142 2013a).

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

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

163 Similar results were obtained when 1% chitosan was applied 3 days before harvest, as it
164 reduced the incidence of postharvest disease in sweet cherries to the same level as the
165 commercially applied synthetic fungicide fenhexamid (Feliziani et al., 2013b). Chitosan
166 (1%) application 7 days before harvest and postharvest hypobaric treatments at 0.25 atm
167 or 0.50 atm for 4 h showed synergistic effects in the control of total rot diseases in
168 sweet cherries stored at 0 °C for 14 days, and thereafter held at 20 °C for a 7-day shelf
169 life (Romanazzi et al., 2003).

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

176

177 *EFFECT OF POSTHARVEST CHITOSAN APPLICATION ON DISEASE CONTROL*

178

179 Use of chitosan for postharvest disease control in temperate fruit was investigated in the
180 1990s in many studies. These studies concerned the application of chitosan in general or
181 focused in a group of chitosans, such as oligochitosan that are characterized by low
182 molecular weight. El Ghaouth et al. (1991a; 1992a) and Zhang and Quantick (1998)
183 reported that the control of gray mold and *Rhizopus* rot in chitosan-coated strawberries
184 was similar to synthetic fungicide application. *Cladosporium* spp. and *Rhizopus* spp.
185 infections were also reported to decrease in artificially inoculated strawberry fruit
186 coated with chitosan and stored at 4 °C to 6 °C for 20 days (Park et al., 2005). Similar

187 results were obtained for table grapes, as small bunches dipped in 0.5% and 1%
188 chitosan solutions, and thereafter artificially inoculated with a *B. cinerea* conidial
189 suspension (by spraying), and stored at low (0 °C) or room (20 °C) temperatures. The
190 chitosan treatment decreased the spread of gray mold infection from one berry to the
191 other berries (nesting) (Romanazzi et al., 2002). Li and Yu (2001) reported that 0.5%
192 and 0.1% chitosan significantly reduced the incidence of brown rot caused by *Monilinia*
193 *fructicola* in peach stored at 23 °C, compared to the untreated fruit. Similarly,
194 application of 1% chitosan reduced postharvest diseases of sweet cherry (Feliziani et al.,
195 2013b). Treatments with chitosan and oligochitosan reduced disease incidence caused
196 by *Alternaria kikuchiana* and *Physalospora piricola* and inhibited lesion expansion of
197 the pear fruit stored at 25 °C. These disease-control effects of chitosan and
198 oligochitosan were concentration dependent and weakened over the incubation time.
199 Indeed, at the lowest chitosan concentration, its effectiveness was the lowest for disease
200 control especially after 5 days of storage at ambient temperatures, compared to the
201 beginning of storage (Meng et al., 2010a). For vegetables such as tomatoes, the
202 infection diameter caused by *R. stolonifer* was 15% less than for the control when
203 treated with chitosan at 1.0%, 1.5% and 2.0%, regardless of the molecular weight
204 (Bautista-Baños and Bravo-Luna, 2004).

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

210 Chitosan has been applied in combination with various biocontrol agents, such
211 as *Candida satoiana* or *Cryptococcus laurentii*, which are microorganisms that have
212 antagonistic actions against postharvest pathogens (El-Ghaouth et al., 2000; De
213 Capdeville et al., 2002; Yu et al., 2007; 2012; Meng et al., 2010b). Spraying of the
214 antagonistic yeast, *C. laurentii*, followed by postharvest chitosan coating significantly
215 reduced the natural decay of table grapes stored at 0 °C. The chitosan coating enhanced
216 the effectiveness of the preharvest spray (Meng et al., 2010b). *C. laurentii* associated
217 with 0.5% chitosan and calcium chloride was effective for the reduction of postharvest
218 blue mold caused by *Penicillium expansum* in pear as well. This combination resulted in
219 more effective mold control than chitosan or *C. laurentii* alone, although chitosan at
220 0.5% inhibited the growth of the biocontrol yeast *in vitro* and *in vivo*. Moreover, after 6
221 days of incubation, the combined treatment with *C. laurentii*, chitosan and calcium
222 chloride inhibited mold decay by nearly 89%, which was significantly higher than the
223 treatments with *C. laurentii*, chitosan or calcium chloride alone, and with the
224 combinations of *C. laurentii* and chitosan, and *C. laurentii* and calcium chloride (Yu et
225 al., 2012). The combination of chitosan and *C. laurentii* on apple resulted in synergistic
226 inhibition of blue mold rot, which was the most effective treatment at the optimal
227 concentration of 0.1% chitosan (Yu et al., 2007). In tropical fruit, the application of the
228 bacterium *Lactobacillus plantarum* alone or in combination with 2% chitosan preserved
229 the quality characteristics of rambutan fruit (Martínez-Castellanos et al., 2009).
230 Similarly, the combination of *Candida satoiana* with 0.2% glycolchitosan was more
231 effective in controlling gray and blue mold of apple and green mold caused by
232 *Penicillium digitatum* of oranges and lemons than the yeast or glycolchitosan alone (El-
233 Ghaouth et al., 2000). On the contrary, the combination of chitosan with *C. satoiana* 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 nutraceutical 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 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

258 cinnamon extract reduced the effectiveness of chitosan in the control of banana crown
259 rot caused by a fungal complex, *Colletotrichum musae*, *Fusarium* spp. and
260 *Lasiodiplodia theobromae* and in delaying fruit senescence during storage (Win et al.,
261 2007). Treatments of papaya with 0.5% or 1.5% chitosan, or with the combination of
262 1.5% chitosan with an aqueous extract of papaya seed, controlled the development of
263 anthracnose diseases of fruit inoculated with *Colletotrichum gloeosporioides*. However,
264 no synergistic effects were obtained with the combination of chitosan at 1.5% and the
265 aqueous extract of papaya for the control of the fungal growth (Bautista-Baños et al.,
266 2003). Similarly, limited control of *R. stolonifer* was observed for chitosan-coated
267 tomatoes in combination with beeswax and lime essential oils (Ramos-García et al.,
268 2012).

269 In some trials chitosan was combined with oleic acid. Coatings based on
270 chitosan either without or with oleic acid at different percentages delayed the
271 appearance of natural fungal infections in comparison to uncoated strawberries. When
272 oleic acid was added to the chitosan coating, there were fewer signs of fungal infection
273 during strawberry storage, especially when the coatings contained the higher levels of
274 oleic acid, which enhanced the antimicrobial properties of chitosan (Vargas et al.,
275 2006).

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

282 stored fruit. Moreover, the presence of chitosan coating prevented the occurrence of
283 heat damage on the fruit surface (Shao et al., 2012). In another investigation, the
284 development of postharvest brown rot on peaches and nectarines was controlled through
285 the heating of fruit to 50 °C for 2 h under 85% relative humidity, which eradicated pre-
286 existing *Monilinia* spp. infections that came from the field, with the application of 1%
287 chitosan at 20 °C then protecting the fruit during handling in the packaging houses and
288 until consumer use (Casals et al., 2012). The combination of immersion in hot water
289 (46.1 °C for 90 min) and in 2% chitosan was beneficial to the storage qualities of
290 mango, compared to untreated mangoes or to fruit treated only with hot water or
291 chitosan (Salvador-Figueroa et al., 2011). Sweet cherries dipped in 1% chitosan and
292 exposed soon after to hypobaric treatment (0.50 atm for 4 h) showed significant
293 reductions in postharvest natural brown rot, gray mold, and total rot diseases, in
294 comparison with the control and with each treatment applied alone. This combination
295 produced a synergistic effect in its reduction of brown rot and total rots (Romanazzi et
296 al., 2003). Chitosan was also applied as a technology to improve benefits obtained with
297 modified atmosphere packaging. The combination of chitosan coating and modified
298 atmosphere packaging was effective in preventing decay and browning, and in retaining
299 the pericarp color in litchi fruit (De Reuck et al., 2009).

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

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

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

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

330 hydrochloric, lactic, L-glutamic, phosphoric, succinic, and L-ascorbic acids. The least
331 effective treatments were chitosan dissolved in maleic or malic acids (Romanazzi et al.,
332 2009).

333

334 *MODE OF ACTION OF CHITOSAN AGAINST THE POSTHARVEST PATHOGENS*

335

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

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

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

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

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

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

402 showed coagulation in the fungus cytoplasm that was characterized by the appearance
403 of small vesicles in the mycelia treated with chitosan. In other cases, the mycelia
404 contained larger vesicles, or even empty cells, which were devoid of cytoplasm (Ait
405 Barka et al., 2004). The area and the elliptical form of the spores was significantly
406 different when *C. gloeosporioides* was grown on potato dextrose agar with added
407 chitosan, compared to potato dextrose agar alone (Bautista-Baños et al., 2003).
408 Similarly, the hyphal and germ-tube morphology of *C. gloeosporioides* growing on
409 chitosan showed malformed hyphal tips with thickened walls. Many swellings occurred
410 in the hyphae or at their tips, whereas in the controls cells the walls and germ tubes
411 were smooth with no swellings or vacuolation (Ali and Mahmud, 2008; Ali et al.,
412 2010). The scanning electron micrographs showed normal growth of hyphae in the
413 untreated controls for *C. gloeosporioides*, whereas there was hyphal agglomeration and
414 formation of large vesicles in the mycelia in samples treated with chitosan-loaded
415 nanoemulsions (Zahid et al., 2012). The fungal mycelia of *Sclerotinia sclerotiorum*
416 exposed to chitosan were deformed, twisted and branched, or indeed, dead, with no
417 visible cytoplasm in the fungal cells, whereas the untreated mycelia were normal in
418 appearance (Cheah et al., 1997).

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

450 the range of 50 kDa to 1000 kDa (Li et al., 2008). The highest inhibitory effect against
451 the growth of *R. stolonifer* was observed with low molecular weight chitosan, while the
452 high molecular weight chitosan showed a greater effect on the development of the
453 spores (Hernández-Lauzardo et al., 2008). High molecular-weight chitosan had the
454 lowest inhibitory effects on *B. cinerea* growth, compared to the low molecular weight
455 chitosan (Badawy and Rabea, 2009). In the case of *S. sclerotiorum*, there was a negative
456 correlation between mycelial growth inhibition and chitosan molecular weight
457 (Ojaghian et al., 2013). Spore germination and germ-tube elongation of *A. kikuchiana*
458 and *P. piricola* were significantly inhibited by chitosan and oligochitosan, although
459 when compared to chitosan, oligochitosan was more effective for the inhibition of spore
460 germination (Meng et al., 2010a). However, other investigations have shown fungal
461 growth inhibition by chitosan, regardless of the type of chitosan (Chien and Chou,
462 2006), without any fungicidal or fungistatic patterns among low, medium, and high
463 molecular weight chitosans tested with different isolates of *C. gloeosporioides*
464 (Bautista-Baños et al., 2005) and *R. stolonifer* (Guerra-Sánchez et al., 2009).

465

466 *INDUCTION OF RESISTANCE BY CHITOSAN IN FRUIT TISSUES*

467

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

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

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

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

497 Chitinase and β -1,3-glucanase are two PR proteins that participate in defense
498 against pathogens, as these can partially degrade the fungal cell wall (Van-Loon and
499 Van-Strien, 1999). Increases in the activities of chitinase and β -1,3-glucanase were
500 demonstrated as a result of chitosan application in ‘Valencia’ oranges, 24 h after the
501 chitosan treatment. It was proposed that these changes in the enzyme activities might
502 have contributed to the reduction of black spot in the orange fruit (Canale Rappussi et
503 al., 2009). Similarly, chitosan coating significantly reduced the decay of strawberry and
504 raspberry, and induced a significant increase in chitinase and β -1,3-glucanase activities
505 of the berries, as compared to the controls (Zhang and Quantick, 1998; Landi et al.,
506 2014). Compared to the untreated fruit, the high chitinase and β -1,3-glucanase activities
507 in chitosan-treated strawberries reinforced the microbial defense mechanism of the fruit
508 and accentuated the resistance against fungal invasion (Zhang and Quantick, 1998;
509 Wang and Gao, 2012). The chitinase and β -1,3-glucanase activities of papaya and
510 mango subjected to chitosan treatment were much higher than in the untreated fruit
511 (Jitareerat et al., 2007; Hewajulige et al., 2009), and oligochitosan treatment
512 significantly enhanced the activities of chitinase and β -1,3-glucanase in pear fruit
513 (Meng et al., 2010a). In table grapes, preharvest chitosan treatments from three different
514 commercial formulations induced the activity of endochitinase, while two of the
515 chitosan formulations induced exochitinase activity (Feliziani et al., 2013a).

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

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

530 Chitosan treatment might induce fruit disease resistance through regulation of
531 ROS levels, antioxidant enzymes, and the ascorbate–glutathione cycle. ROS, such as
532 H₂O₂ and O₂⁻, are the earliest events that correlate plant resistance to pathogens (Baker
533 and Orlandi, 1995); these are involved in the development of disease resistance in fruit
534 (Torres et al., 2003). Although ROS might contribute to an enhancement of the plant
535 defense, high level of ROS can cause lipid peroxidation and lead to the loss of
536 membrane integrity of plant organs. To prevent harmful effects of excess ROS on plant
537 tissues, the ROS can be detoxified by an antioxidant system. This consists of non-
538 enzymatic antioxidants, such as ascorbic acid, glutathione, and phenolic compounds,
539 and antioxidant enzymes, such as superoxide dismutase, peroxidases and catalases.
540 Chitosan application was reported to reduce ROS in tissues of treated fruit, such as pear
541 (Li et al., 2010a) and guava (Hong et al., 2012), and to lower the hydrogen peroxide
542 content in litchi (Sun et al., 2010), pear (Li et al., 2010a), table grapes (Feliziani et al.,
543 2013a) and strawberry (Romanazzi et al., 2013). This might be due to direct effects, as

544 chitosan itself has antioxidant activity and scavenges hydroxyl radicals (Yen et al.,
545 2008), or to indirect effects, as chitosan induces the plant antioxidant system.

546 Higher levels of glutathione were reported after chitosan treatment in litchi (Sun
547 et al., 2010), strawberry (Wang and Gao, 2012) and orange (Zeng et al., 2010). Higher
548 quantities of ascorbic acid have also been reported after chitosan treatments in fruit
549 tissues of strawberry (Wang and Gao, 2012), peach (Li and Yu, 2001; Ruoyi et al.,
550 2005), sweet cherry (Dang et al., 2010; Kerch et al., 2011), jujube (Qiuping and
551 Wenshui, 2007; Xing et al., 2011b), orange (Zeng et al., 2010), citrus (Chien and Chou,
552 2006), longan (Jiang and Li, 2001), guava (Hong et al., 2012), mango (Jitareerat et al.,
553 2007; Zhu et al., 2008) and litchi (Sun et al., 2010). The reduction of ascorbic acid loss
554 in chitosan-coated sweet cherries was proposed to be due to the low oxygen
555 permeability of the chitosan coating around the fruit surface, which lowers the oxygen
556 level and reduces the activity of the ascorbic acid oxidase enzymes, which prevents the
557 oxidation of ascorbic acid (Dang et al., 2010).

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

568 chitosan had higher phenolic compound contents (Shiri et al., 2012; Feliziani et al.,
569 2013a). Anthocyanin, flavonoid and total phenolics contents of chitosan treated litchi
570 decreased more slowly than in untreated fruit (Zhang and Quantick, 1997; Jiang et al.,
571 2005; De Reuck et al., 2009). Kerch et al. (2011) reported that total phenols and
572 anthocyanin content increased in chitosan-treated sweet cherry after 1 week of cold
573 storage, while their contents decreased in chitosan-treated strawberry stored under the
574 same conditions. Similarly, in strawberry, chitosan-coated fruit had lower anthocyanin
575 content, as the anthocyanins were synthesized at a slower rate than for the non-treated
576 berries (El Ghaouth et al., 1991a), and the rate of pigment development was lower with
577 an increase in chitosan concentration (Reddy et al., 2000a). The anthocyanin contents
578 significantly decreased throughout storage in strawberries coated with chitosan
579 combined with oleic acid, whereas no significant changes were seen in the control
580 samples at the end of the storage (Vargas et al., 2006). On the contrary, Wang and Gao
581 (2012) reported that strawberries treated with chitosan maintained better fruit quality,
582 with higher levels of phenolics, anthocyanins and flavonoids. In another study, the
583 application of chitosan to strawberry increased the expression of genes involved in the
584 biosynthesis of flavonoid compounds, such as chalcone isomerase, flavonol synthase,
585 anthocyanidin synthase (Landi et al., 2014). Several factors, such as the cultivar of the
586 studied commodity, the stage of maturation, the storage conditions, could account to
587 explain the different responses to chitosan application concerning phenolic compounds
588 accumulation in fruit tissues.

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

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

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

616 great impact on fruit quality; indeed, PPO is a phenol-related metabolic enzyme that
617 catalyzes the oxidation of phenolic compounds that are involved in plant defense
618 against biotic and abiotic stresses and in pigmentation/ browning of fruit and vegetable
619 tissues (Lattanzio et al., 2006; Bhattacharya et al., 2010). In some investigations,
620 chitosan decreased PPO activity, and its inhibitory effects are probably a consequence
621 of the adsorption of suspended PPO, its substrates, or its products by the positive
622 charges of chitosan (Badawy and Rabea, 2009). The other possibility is that the
623 selective permeability to gases due to the chitosan coating generates low levels of
624 oxygen around the fruit surface, which can delay the deteriorative oxidation reactions,
625 and partially inhibit the activities of oxidases such as PPO (Ayranci and Tunc, 2003).
626 The chitosan coating markedly reduces PPO activity and delays skin browning during
627 fruit shelf life. The maintenance of the skin color of the litchi fruit after chitosan
628 treatment can be accounted for by the higher level of anthocyanin content in the skin
629 that results from inhibition of PPO activity (Zhang and Quantick, 1997; Jiang et al.,
630 2005; De Reuck et al., 2009). Similarly, the activities of PPO and peroxidase, and the
631 related browning in the pericarp, were markedly lowered by treatment of harvested
632 litchi fruits with ascorbic acid and 1% chitosan (Sun et al., 2010). In chitosan-treated
633 tomato (Badawy and Rabea, 2009) and jujube (Wu et al., 2010; Xing et al., 2011b), the
634 decreases in the PPO activities were concomitant with the enhanced phenolic content,
635 and in sweet cherry (Dang et al., 2010), with the reduction in tissue browning. The
636 combination of chitosan, calcium chloride and intermittent warming decreased the PPO
637 activity in the tissues of peach that had been cold stored for 50 days (Ruoyi et al., 2005).
638 However, in other investigations, PPO activities of fruit tissue increased after chitosan
639 treatment. Chitosan treatment enhanced the activities of PPO in the flesh around the

640 wound of a pear (Meng et al., 2010a). An increase in the activity of PPO was
641 demonstrated as a result of chitosan application in ‘Valencia’ oranges, which was seen
642 24 h after chitosan treatment (Canale Rappussi et al., 2009). Chitosan application in
643 tomato fruit stored at 25 °C and 2 °C increased the content of the phenolic compounds
644 and induced the activities of PPO, the levels of which were almost 1.5-fold those in the
645 wounded control fruit at the same time (Liu et al., 2007). In this study, there was no
646 direct relationship between the PPO activities and the content of phenolic compounds,
647 although the phenolic compounds can be oxidized by the actions of PPO and
648 peroxidase, to produce quinones (Campos-Vargas and Saltveit, 2002). It is likely that
649 regulation of phenolic metabolism by the action of other enzymes, such as PAL, which
650 participates in the biosynthesis of phenolic compounds, also has an important role (Liu
651 et al., 2007). This could even explain the reason why in some investigations the PPO
652 levels of fruit tissue after chitosan application are variable. Preharvest spraying with *C.*
653 *laurentii* combined with postharvest chitosan coating increased the activities of PPO in
654 table grapes during storage, but after 3 days of shelf life, the PPO activities in the
655 treated fruit were lower than in the untreated fruit (Meng et al., 2010b). During cold
656 storage, the PPO activity of litchi fruit coated with chitosan increased slowly, reached a
657 peak, and then decreased (Zhang and Quantick, 1997).

658

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

661

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

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

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

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

690 Chitosan forms a coating film on the outside surface of sweet cherries that
691 effectively delayed the loss of water and promoted changes in titratable acidity and total
692 soluble solids of the sweet cherries (Dang et al., 2010). Strawberries treated with
693 chitosan alone or in combined with calcium gluconate showed reduced weight loss and
694 respiration, which delayed the ripening and the progression of fruit decay due to
695 senescence. Regardless of the addition of calcium gluconate to the chitosan, the coated
696 strawberries had higher titratable acidity, and lower pH and soluble solids (Hernández-
697 Muñoz et al., 2008). A chitosan coating without or with added calcium or vitamin E
698 decreased weight loss and delayed the changes in pH and titratable acidity of
699 strawberries and red raspberries during cold storage (Han et al., 2004; 2005). Chitosan
700 application combined with bergamot oil provided a water vapor barrier for cold-stored
701 table grapes, which reduced the fruit weight losses. Due to its hydrophobic nature, the
702 addition of bergamot oil lowered this phenomenon further (Sánchez-González et al.,
703 2011). Similarly, weight loss reductions in chitosan-coated table grapes were observed
704 when this was combined with putrescine (Shiri et al., 2012) and grape seed extract (Xu
705 et al., 2007b). The complex of zinc(II) and cerium(IV) with chitosan film-forming
706 material that was applied to preserve the quality of Chinese jujube fruit reduced the fruit
707 respiration rate and weight loss, while it increased the fruit total soluble solids, as
708 compared to the uncoated fruit (Wu et al., 2010). In another study, after 42 days of
709 storage at 13 °C, chitosan-coated citrus fruit showed less weight loss and higher
710 titratable acidity and total soluble solids, compared to the control fruit. The weight loss
711 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 CO₂ and decreased the internal O₂ 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

736 weight, and titratable acidity were all effectively inhibited by chitosan (Jitareerat et al.,
737 2007), while the increase in total soluble solids was delayed during storage (Zhu et al.,
738 2008). Mango fruit coated with chitosan and subjected to hydrothermal treatment had
739 less weight loss, lower pH and soluble solids, but higher acidity, regardless of the
740 hydrothermal process (Salvador-Figueroa et al., 2011). The CO₂ concentration in the
741 internal cavity of chitosan-treated papaya was significantly higher than that of the
742 untreated fruit. The formation of a chitosan film on the fruit acted as a barrier for O₂
743 uptake, and slowed the rate of respiration and the metabolic activity, and consequently
744 the ripening process (Hewajulige et al., 2009). Again in papaya, chitosan provided
745 effective control of weight loss, and delayed the changes in soluble solids
746 concentrations over 5 weeks of storage. The titratable acidity of the papaya fruit
747 declined throughout the storage period, although at a slower rate in the chitosan-coated
748 fruit, as compared to the untreated fruit (Bautista-Baños et al., 2003; Ali et al., 2010;
749 2011). Chitosan coating without or with calcium infiltration markedly slowed the
750 ripening of papaya, as shown by their lack of weight loss, delay in titratable acidity
751 decrease, and increase in soluble solids and pH (Al Eryani et al., 2008). In litchi fruit
752 during storage, chitosan treatment produced an effective coating that reduced the
753 respiration and transpiration of the fruit during storage (Lin et al., 2011), and reduced
754 the decreases in the concentrations of total soluble solids and in the titratable acidity
755 (Jiang et al., 2005). Similar results were obtained with the combination of chitosan with
756 ascorbic acid, which significantly increased the titratable acidity and total soluble solids
757 of stored litchi fruit (Sun et al., 2010).

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

760 deterioration of the cell-wall composition, which involves the hydrolysis of pectin by
761 enzymes; e.g., polygalacturonase (Atkinson et al., 2012). Low levels of oxygen and
762 higher levels of carbon dioxide restricts the activities of these enzymes and promotes
763 the retention of fruit firmness during storage (Maqbool et al., 2011). Moreover, due to
764 reduced transpiration, the water retention provides turgor to the fruit cells. Banana fruit
765 treated with composite edible coatings of chitosan and Arabic gum showed significantly
766 higher firmness than untreated bananas at the end of the storage period, and this
767 firmness decreased as the concentration of the coating decreased (Maqbool et al., 2011).
768 Chitosan coatings had beneficial effects on strawberry firmness, such that by the end of
769 the storage period, the treated fruit had higher flesh firmness values than the untreated
770 fruit (Hernández-Muñoz et al., 2008). In several other studies, chitosan coating
771 maintained the firmness during storage of table grapes (Xu et al., 2007b; Sánchez-
772 González et al., 2011), apple (Shao et al., 2012), pear (Lin et al., 2008), peach (Li and
773 Yu, 2001), jujube (Qiuping and Wenshui, 2007), orange (Chien and Chou, 2006; Cháfer
774 et al., 2012), banana (Kittur et al., 2001; Win et al., 2007; Baez-Sañudo et al., 2009),
775 mango (Zhu et al., 2008; Salvador-Figueroa et al., 2011), papaya (Bautista-Baños et al.,
776 2003; Sivakumar et al., 2005b; Ali et al., 2010; 2011), rambutan (Martínez-Castellanos
777 et al., 2009), guava (Hong et al., 2012) and tomato (El Ghaouth et al., 1992b) (Tables 6-
778 8).

779 In several studies, panelists were asked to observe and then rate the overall
780 appearance, or just the flavor, of fruit treated or not with chitosan, using hedonic scales
781 (Tables 6-8). These studies showed that chitosan can preserve the taste of pear fruit,
782 which after cold storage was similar to the taste of the fresh fruit (Zhou et al., 2008).
783 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

808 about 1 month of storage, developed poor pulp color and inferior texture, and were off-
 809 flavored (Maqbool et al., 2011). Similarly, the sensory evaluation of papaya for taste,
 810 peel color, pulp color, texture, and flavor revealed that the fruit treated with 1.5%
 811 chitosan attained maximum scores from the panelists in all of the tested parameters. The
 812 untreated fruit and those treated with 0.5% chitosan ripened after 3 weeks of storage,
 813 and then began to decompose, while the fruit treated with 2% chitosan did not ripen
 814 fully after more than 1 month of cold storage. This was because of the thickness of the
 815 chitosan coating, which blocked the lenticels and caused fermentation inside, and in
 816 both cases the fruit were discarded from the evaluation due to the unacceptable quality.
 817 The flavor of the fruit with 1.5% chitosan coating was rated as excellent, because the
 818 pulp was not only sweet and pleasant, but also had a characteristic aroma (Ali et al.,
 819 2010; 2011). Litchi fruit subjected to chitosan treatment either alone or combined with
 820 carbonate salts showed good eating quality (Sivakumar et al., 2005a).

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

832 papaya fruit, as noted from the lower values of lightness and chroma and the higher
833 value of hue angle in treated papaya, compared to untreated papaya (Al Eryani et al.,
834 2008). During storage, chitosan coating delayed color changes in banana (Kittur et al.,
835 2001; Win et al., 2007; Baez-Sañudo et al., 2009; Maqbool et al., 2011), litchi fruit
836 (Zhang and Quantick, 1997; Caro and Joas, 2005; Joas et al., 2005; Ducamp-Collin et
837 al., 2008; De Reuck et al., 2009; Sun et al., 2010), mango (Zhu et al., 2008; Salvador-
838 Figueroa et al., 2011), citrus (Canale Rappussi et al., 2011), strawberry (Han et al.,
839 2004; 2005; Hernández-Muñoz et al., 2008), and tomato (El Ghaouth et al., 1992b).
840 Sensory analyses also revealed beneficial effects of chitosan coating in terms of
841 delaying rachis browning and maintenance of the visual aspects of table grape berries
842 (Xu et al., 2007b; Sánchez-González et al., 2011).

843 Fruit and vegetables treated with chitosan have a higher nutritional value,
844 because chitosan can retain the contents of the ascorbic and phenolic compounds
845 (Tables 6-8), which are positively correlated with antioxidant capacity (Rapisarda et al.,
846 1999). Moreover, chitosan can be used as a vehicle for the incorporation of functional
847 ingredients, such as other antimicrobials, minerals, antioxidants and vitamins. Some of
848 these combinations can enhance the effects of chitosan or reinforce the nutritional value
849 of the commodities (Vargas et al., 2008). Chitosan-based coatings can also carry high
850 concentrations of calcium or vitamin E, thus significantly increasing the content of these
851 nutrients in fresh and frozen strawberry and raspberry. Incorporation of calcium or
852 vitamin E into chitosan-based coatings did not alter its antifungal properties, while it
853 enhanced the nutritional value of these fresh and frozen strawberry and raspberry (Han
854 et al., 2004). In addition, incorporation of calcium chloride in chitosan coating increased
855 the stability of the cell wall and middle lamella of the strawberry tissue, and improved

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

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

880 2004), such as ZnO (Li et al., 2010b) or Ag (Pinto et al., 2012) nanoparticles, improved
881 the mechanical and barrier properties (Pereira de Abreu et al., 2007) and the thermal
882 stability of the films (de Moura et al., 2009).

883

884 *EFFECTS OF CHITOSAN ON FOODBORNE PATHOGENS*

885

886 Foodborne illnesses are diseases that are caused by agents that enter the human body
887 through the ingestion of food. In 2011, the Center for Disease Control and Prevention
888 (CDC) estimated that in the United States each year there are 48 million foodborne
889 illnesses that are responsible for 128,000 hospitalizations and 3,000 deaths (CDC,
890 2011). The World Health Organization (WHO) estimates that in 2005, 1.5 million
891 people died worldwide from diarrheal diseases, with a great proportion of these cases
892 being foodborne (WHO, 2006). Furthermore, in the future, with the growth of
893 populations and movement of goods and people at the global scale, this might make the
894 control of foodborne infections more difficult.

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

904 contaminated by these microorganisms during the preharvest stage, mainly by
905 contaminated water or sewage and faeces, or during the postharvest stage, in the
906 handling and storage of the horticultural products. The growth of microorganisms on
907 fresh-cut produce can also occur during the cutting and slicing operations (Beuchat,
908 2002).

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

913 Inatsu et al. (2010) evaluated different sanitizers to prevent growth of four
914 strains of *E. coli* on the surface of tomato fruit, and they found that 0.1% chitosan was
915 effective when applied after a sodium chloride washing treatment. However, in this
916 case, other combinations of sanitizers were more effective (e.g., 0.1% lactic acid with
917 0.05% sodium chloride). Chitosan coating reduced the native microflora on the surface
918 of litchi fruit (Sivakumar et al., 2005a) and strawberry (Ribeiro et al., 2007), but not for
919 table grapes (Romanazzi et al., 2002). However, several additives can be incorporated
920 into the chitosan coating, which can provide more specific functions, such as
921 antimicrobial activity that is aimed at either preventing or reducing the growth of
922 foodborne microorganisms (Vargas et al., 2008). Coatings of chitosan and allyl
923 isothiocyanate on cantaloupe reduced the *Salmonella* presence down to the limit of
924 detection after 2 weeks of storage (Chen et al., 2012). Also when recontamination of
925 cantaloupe with *Salmonella* was simulated, the results indicated that the chitosan-allyl
926 isothiocyanate coating not only reduced the *Salmonella* more than the current practice
927 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 DH5 α 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.

976 According to several studies, Gram-positive bacteria are more susceptible to chitosan
977 than Gram-negative bacteria (No et al., 2002; Takahashi et al., 2008; Jung et al., 2010;
978 Tayel et al., 2010), while according to others, the opposite is the case (Devlieghere et
979 al., 2004). A recent study reported the effectiveness of chitosan and its derivatives
980 against well-established biofilms formed by foodborne bacteria, which are assumed to
981 be resistant to cleaning and disinfection practices. The results showed that a 1 h
982 exposure to chitosan resulted in reductions in viable cells on mature *L. monocytogenes*
983 biofilms, and in the attached populations of the other organisms tested, as *B. cereus*,
984 *Salmonella enterica* and *Pseudomonas fluorescens*, except for *S. aureus* (Orgaz et al.,
985 2011).

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

1000 would also be expected to have stronger antibacterial activities (Jung et al., 2010; Kong
1001 et al., 2010; Tayel et al., 2010). On the other hand, numerous studies have generated
1002 different results relating to correlations between the chitosan bactericidal activities and
1003 its molecular weight. In some studies, lower molecular weight chitosans (ranging from
1004 2.7×10^4 to 5.5×10^4 Da) was more effective against bacteria than higher molecular
1005 weight chitosans (Liu et al., 2006; Tayel et al., 2010; Kim et al., 2011). In other studies,
1006 this trend was observed against Gram-negative bacteria, but not against Gram-positive
1007 bacteria (No et al., 2002; Zheng and Zhu, 2003). According to Benhabiles et al. (2012),
1008 when the molecular weight of chitosan is low, its polymer chains have greater flexibility
1009 to create more bonds, and they can thus better interact with the microbial cells. In other
1010 studies, no trends were reported for the antibacterial actions related to increased or
1011 decreased molecular weights of chitosan (Jung et al., 2010; Mellegård et al., 2011).

1012

1013 *CONCLUSIONS AND FUTURE TRENDS*

1014

1015 This review reports on the recent and most relevant studies concerning preharvest
1016 spraying and postharvest application of chitosan for fruit and vegetables. These studies
1017 have shown that this biopolymer can effectively maintain the fruit and vegetable
1018 quality, and can control their postharvest decay during storage and shelf life. Studies
1019 dealing with the mechanisms of action of chitosan as an antimicrobial against
1020 postharvest fungi and foodborne bacteria are also summarized here. The film-forming
1021 properties, antimicrobial activities, and induction of plant resistance of chitosan appear
1022 to be the main factors in its success. With its intrinsic properties, and because of its
1023 double activity on the host and the pathogen, chitosan can be considered as the first of a

1024 new class of plant-protection products (Bautista-Baños et al., 2006). Moreover, chitosan
1025 has been under considerable investigation for applications in biomedicine,
1026 pharmacology, biotechnology, and in the food industry, due to its biocompatibility,
1027 biodegradability, and bioactivity (Synowiecki and Al-Khateeb, 2003; Tharanathan and
1028 Kittur, 2003; Wu et al., 2005). Chitosan is not toxic to humans and its safe use as a
1029 pharmaceutical carrier has been reported (Baldrick, 2010; USFDA, 2013).

1030 Chitosan has been reported to be a potentially viable alternative for fruit and
1031 vegetable preservation. Multicomponent edible coatings can be produced with suitable
1032 ingredients for the product to provide the desired barrier protection, while also serving
1033 as a vehicle for the incorporation of specific additives that can enhance the
1034 functionality, such as antioxidants and antimicrobials, thus avoiding pathogen or
1035 foodborne microorganism growth on the surface of fruit and vegetable products
1036 (Valencia-Chamorro et al., 2011). The combination of chitosan with minerals, vitamins
1037 or other nutraceutical compounds can reinforce the nutritional value of the commodities,
1038 without reducing the taste acceptability. This new generation of edible coatings is being
1039 especially designed to allow incorporation and/or controlled release of antioxidants,
1040 vitamins, nutraceuticals, and natural antimicrobial agents (Vargas et al., 2008;
1041 McClements et al., 2009).

1042 The availability of commercial chitosan products that are easily dissolvable in
1043 water now provides an alternative to synthetic fungicides for growers, for the control of
1044 diseases of fruit and vegetables. However, at present, none of the formulations of
1045 chitosan are registered as plant protectant products. The present review summarizes the
1046 application of chitosan either preharvest or postharvest. Here, postharvest treatment is
1047 not advisable for fruit that are characterized by a bloom on the surface, such as table

1048 grapes, or that have a thin waxy pericarp and succulent flesh, such as strawberries,
1049 which can be easily damaged during harvest and postharvest handling. On these
1050 commodities, preharvest treatment (even 1-2 days before harvest) can be considered as
1051 a promising approach to control the postharvest decay of these fruit under storage.
1052 Although a lot of information regarding the effectiveness of chitosan in the control of
1053 postharvest decay of fruit and vegetables is available, its application to large-scale tests
1054 and its integration into commercial agricultural practices are key points that need to be
1055 investigated further. In addition, more studies concerning the exact mechanisms of
1056 action of chitosan are needed. Also, several mechanisms relating to its antifungal and
1057 antibacterial activities remain unclear. New knowledge about these aspects will provide
1058 the necessary information to support decisions relating to the preparation of the
1059 chitosan, which molecular weight chitosan to use, and the kind of commercial
1060 formulation.

1061

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- 1698

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	<i>Cryptococcus laurentii</i>	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, <i>Candida satoiana</i> , harpin <i>Cryptococcus laurentii</i> <i>Candida satoiana</i> Heat treatment	De Capdeville et al., 2002 (postharvest) Yu et al., 2007 (postharvest) El Ghaout et al., 2000 (postharvest) Shao et al., 2012 (postharvest)
	Gray mold	<i>Candida satoiana</i> Heat treatment	El Ghaout et al., 2000 (postharvest) Shao et al., 2012 (postharvest)
Pear	Blue mold	Calcium chloride + <i>Cryptococcus laurentii</i>	Yu et al., 2012 (postharvest)
Peach	Brown rot	- Heat treatment	Li and Yu, 2001 (postharvest) 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)

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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
<i>Alternaria alternata</i>	Tomato	Sánchez-Domínguez et al., 2011
<i>Alternaria kikuchiana</i>	Pear	Meng et al., 2010a
<i>Aspergillus phoenicus</i>	Pear	Cè et al., 2012
<i>Aspergillus niger</i>		Plascencia-Jatomea et al., 2003
<i>Botrydiploia lecanidion</i>	Tankan citrus fruit	Chien and Chou, 2006
<i>Botrytis cinerea</i>	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
<i>Cladosporium</i> spp.	Litchi fruit, strawberry	Park et al., 2005; Sivakumar et al., 2005a
<i>Colletotrichum gloeosporioides</i>	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
<i>Colletotrichum musae</i>	Banana	Win et al., 2007; Maqbool et al., 2010a; 2010b; Zahid et al., 2012
<i>Colletotrichum</i> spp.	Table grapes and tomato	Muñoz et al., 2009
<i>Fusarium solani</i>		Eweis et al., 2006
<i>Fusarium sulphureum</i>	Potato	Yong-Cai, et al., 2009
<i>Fusarium</i> spp.	Banana	Win et al., 2007
<i>Geotricum candidum</i>		El-Mougy et al., 2012
<i>Guignardia citricarpa</i>	Orange	Canale Rappussi et al., 2009; 2011
<i>Lasioidiplodia theobromae</i>	Banana	Win et al., 2007
<i>Monilinia fructicola</i>	Apple, peach	Yang et al., 2010; 2012
<i>Monilinia laxa</i>	Sweet cherry	Feliziani et al., 2013b
<i>Penicillium citrinum</i>	Jujube	Xing et al., 2011b
<i>Penicillium digitatum</i>	Orange, lemon, Tankan citrus fruit	El Ghaouth et al., 2000; Bautista Baños et al., 2004; Chien and Chou, 2006; El-Mougy et al., 2012
<i>Penicillium expansum</i>	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
<i>Penicillium italicum</i>	Tankan citrus fruit	Chien and Chou, 2006; El-Mougy et al., 2012
<i>Penicillium stolonifer</i>	Pear	Cè et al., 2012
<i>Phytophthora cactorum</i>	Strawberries	Eikemo et al., 2003
<i>Physalospora piricola</i>	Pear	Meng et al., 2010a
<i>Rhizopus stolonifer</i>	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
<i>Sclerotinia sclerotiorum</i>	Carrot	Cheah et al., 1997; Molloy et al., 2004; Ojaghian et al., 2013

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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	- <i>Cryptococcus laurentii</i>	Romanazzi et al., 2002; Meng et al., 2008 Meng and Tian, 2009; Meng et al., 2010b	
	Peroxidase	-	Meng et al., 2008; Gao et al., 2013	
	Polyphenol oxidase, superoxide dismutase	- <i>Cryptococcus laurentii</i>	Meng et al., 2008; Gao et al., 2013 Meng and Tian, 2009; Meng et al., 2010b	
	Chitinase, myricetin	-	Feliziani et al., 2013a	
	Quercetin	-	Feliziani et al., 2013a	
	Respiration	Putrescine	Shiri et al., 2012	
	Trans-resveratrol	Bergamot oil	Sánchez-González et al., 2011	
		UV	Romanazzi et al., 2006	
		-	Feliziani et al., 2013a	
	Soluble solids content	-	Meng et al., 2008	
		Bergamot oil	Sánchez-González et al., 2011	
		<i>Cryptococcus laurentii</i>	Meng et al., 2010b	
		Glucose	Gao et al., 2013	
	Titratable acidity	-	Meng et al., 2008	
	Total phenolic content	-	Meng et al., 2008	
		<i>Cryptococcus laurentii</i>	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
Raspberry	reductase Weigth loss, color, pH, titratable acidity Ascorbic acid, titratable acidity, firmness, antocyanin content	Vitamin E -	Han et al., 2004 Zhang and Quantick, 1998
Apple	Respiration, firmness, weicht loss, titratable acidity	Heat	Shao et al., 2012
Pear	Polyphenol oxidase, chitinase, β -1,3 glucanase, ROS, catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase Peroxidase Respiration, permeability of cell membrane, weight loss	- - Ascorbic acid	Meng et al., 2010b Li et al., 2010a Meng et al., 2010b; Li et al., 2010a Zhou et al., 2008 Lin et al., 2008
Apricot	Soluble solid contents, titratable acidity, firmness	Ascorbic acid	Lin et al., 2008
Peach	Total phenolic content, antioxidant activity, weight loss Titratable acidity, ascorbic acid, respiration, firmness, ethylene and malondialdehyde production, superoxide dismutase Polyphenol oxidase, peroxidase, ascorbic acid oxidase, polygalacturonase, vitamic C	- - CaCl ₂ coating + PEpackage + intermittent warming	Ghasemnezhad et al., 2010 Li and Yu, 2001 Ruoyi et al., 2005
Sweet cherry	Titratable acidity, soluble solid, catalase, peroxidase, polyphenol oxidase, phenilalanine ammonia-lyase, respiration Ascorbic acid	- -	Dang et al., 2010 Dang et al., 2010; Kerch et al., 2011
Orange	Phenols content, antocyanin content Water loss, firmness	- Bergamot, thyme, tea tree essential oil	Kerch et al., 2011 Cháfer et al., 2012
	Color Chitinase, b-1,3-glucanase, polyphenol oxidase Peroxidase	- - -	Canale Rappussi et al., 2011 Canale Rappussi et al., 2009 Canale Rappussi et al., 2009; Zeng et al.,

Fruit	Physiological change	Integration to chitosan	References
	Superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, hydrogen peroxide content, ascorbate content	-	2010 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
	Ascorbic acid	Zinc, cerium	Wu et al., 2010
		-	Xing et al., 2011b
	Firmness	1-methylcyclopropene	Qiuping and Wenshui, 2007
	Weight loss	1-methylcyclopropene	Qiuping and Wenshui, 2007
		1-methylcyclopropene	Qiuping and Wenshui, 2007
		Zinc, cerium	Wu et al., 2010
	Respiration, soluble solids	Zinc, cerium	Wu et al., 2010

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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 Arabic gum	Baez-Sañudo et al., 2009 Maqbool et al., 2010a, 2010b
	Respiration	-	Kittur et al., 2001
		1-methylcyclopropene Arabic gum	Baez-Sañudo et al., 2009 Maqbool et al., 2011
	Firmness, soluble solids content	-	Kittur et al., 2001; Win et al., 2007
		1-methylcyclopropene Arabic gum	Baez-Sañudo et al., 2009 Maqbool et al., 2010a; 2010b; 2011
	Color change	-	Kittur et al., 2001; Win et al., 2007
1-methylcyclopropene Arabic gum		Baez-Sañudo et al., 2009 Maqbool et al., 2011	
Longan fruit	Weight loss	Arabic gum	Maqbool et al., 2010a; 2010b; 2011
	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	
Papaya	Respiration, ascorbic acid	-	Jitareerat et al., 2007; Zhu et al., 2008
	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
Litchi fruit	Weight loss, color change	Calcium infiltration	Al Eryani et al., 2008
		-	Ali et al., 2011
		Ammonium carbonate, sodium bicarbonate	Sivakumar et al., 2005b
	Firmness	Calcium infiltration	Al Eryani et al., 2008
		-	Ali et al., 2010; 2011
		Ammonium carbonate, sodium bicarbonate	Sivakumar et al., 2005b
	Chitinase, b-1,3-glucanase	Aqueous extract of papaya seeds	Bautista-Baños et al., 2003
	Respiration	-	Hewajulige et al., 2009
	Weight loss	-	Hewajulige et al., 2009, Ali et al., 2011
	Titratable acidity	-	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
		-	Jiang et al., 2005; Sivakumar et al., 2005a; Sun et al., 2010
	Total phenolic content, flavonoid content	Organic acids	Joas et al., 2005; Caro and Joas, 2005
	Anthocyanin content	-	Zhang and Quantick, 1997; Sivakumar et al., 2005a
	Respiration rate	-	Zhang and Quantick, 1997; Jiang et al., 2005; Sivakumar et al., 2005a;
Modified atmosphere packaging		De Reuck et al., 2009	
-		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, malondialdehyde; ascorbic acid content	Ascorbic acid	Sun et al., 2010
Rambutan	Firmness, soluble solid, titratable acidity	<i>Lactobacillus plantatum</i>	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

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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	Weight loss, ascorbic acid, pH	Natamycin	Cong et al., 2007
Carrot	Polyphenol oxidase, peroxidase, phenylalanine ammonia-lyase	-	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
<i>Escherichia coli</i>	Tomato	-	Inatsu et al., 2010
	Tomato	Beeswax + lime essential oil	Ramos-García et al., 2012
<i>Salmonella</i> spp.	Whole cantaloupe	Allyl isothiocyanate, nisin	Chen et al., 2012

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