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1 **GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the**
2 **control of postharvest diseases of fresh horticultural produce**

3

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25 **Abstract**

26 Postharvest decay caused by fungal pathogens is one of the most important factors causing
27 economic losses for the worldwide industry of fresh horticultural produce. Despite the positive
28 results of the use of conventional chemical fungicides, alternatives for decay control are needed
29 because of increasing important concerns related to their massive and continued use. Low-toxicity
30 chemical alternatives evaluated for control of postharvest diseases of temperate, subtropical and
31 tropical fruit, and fruit-like vegetables are reviewed in this chapter. These compounds should have
32 suitable antifungal activity while showing known and very low toxicological effects on mammals
33 and impact on the environment. In addition, they should be exempt from residue tolerances on
34 agricultural commodities. Authorities confirm these characteristics by approving them as food
35 additives or preservatives or as generally regarded as safe (GRAS) substances. Among those of
36 synthetic origin, the most important are inorganic or organic salts, e.g. carbonates, sorbates,
37 benzoates, paraben salts, etc., and composite edible coatings formulated with antifungal ingredients.
38 Hydrocolloids (polysaccharides such as cellulose derivatives, alginates, pectins, or gums, and various
39 plant proteins) and food-grade lipids are the main components of the matrix of composite coatings.
40 Interesting antifungal ingredients include GRAS salts, essential oils, and antagonistic
41 microorganisms. Low-toxicity chemicals of natural origin include plant extracts, including essential
42 oils, antifungal peptides and small proteins, and coatings based on chitosan or plant gels like those
43 from *Aloe* spp. Efficacy and overall performance, advantages, disadvantages, limitations, and
44 potential combined use of these chemical alternatives in hurdle technologies for postharvest decay
45 control are discussed.

46

47 **Keywords:** antifungal peptides, antifungal edible coatings, antimicrobial salts, chitosan, food
48 preservatives, plant extracts

49

50 **Highlights**

- 51 • Low-toxicity chemicals alternative to conventional fungicides are reviewed
- 52 • They are food additives or GRAS substances from synthetic or natural origin
- 53 • GRAS inorganic or organic salts include carbonates, sorbates, benzoates
- 54 • Composite edible coatings are formulated with antifungal ingredients
- 55 • Chitosan is the most important natural antifungal edible coating
- 56 • Other natural compounds include plant extracts, antifungal peptides, and *Aloe* spp. coatings

57

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73 **1. Introduction**

74 Fruit and vegetables are readily consumed in fresh or processed form, directing substantial
75 interest towards maintaining quality of fresh produce. The health and nutritional benefits of fruits
76 and vegetables are significant, due to the presence of large amounts of antioxidants and
77 micronutrients (Ramos et al., 2013). Moreover, these products provide an important source of
78 income and employment in producing countries and are important drivers of their economic
79 development (Mohamed et al., 2011). Thus, a lot of investment is focused on reaching the desirable
80 markets and extending shelf life without compromising the quality of the product. There is,
81 therefore, a huge demand for postharvest technologies for handling fresh produce (Yahia et al.,
82 2011). These technologies should offer protection from postharvest diseases as well as over-ripening.
83 Amongst all pathogens, fungal plant pathogens are more prevalent and are one of the major causes of
84 quality deterioration of fruit and vegetables, mainly due to the high moisture content of these food
85 products. Fungal infections typically results in decay, accelerated ripening, as well as accumulation
86 of mycotoxins (Tripathi and Dubey, 2004). To mitigate the infection pressure and properly control
87 disease, producers rely heavily on application of chemical fungicides. Use of fungicides has led to
88 substantial improvements in maintaining the shelf life of fresh produce. Modern fungicides,
89 however, are organic compounds, with a high degree of specificity towards their target organism.
90 Despite the positive results of the use of modern fungicides, increasing concerns continue to be
91 expressed about health hazards and environmental pollution due to the use of large quantities of
92 chemicals (Bautista-Baños et al., 2006; Gupta and Dikshit, 2010). Furthermore, the continuous use of
93 these synthetic compounds has led in many cases to the proliferation of resistant biotypes of fungal
94 pathogens. The build-up of single, double, and even triple-resistant isolates in the populations of
95 fungal pathogens in commercial packinghouses seriously compromises the effectiveness of these
96 chemicals (Palou et al., 2008). Therefore, new approaches for controlling postharvest diseases have
97 shifted towards prioritizing alternatives to synthetic fungicides.

98 Among the alternative methods of different nature for decay control, in this chapter we focus
99 on low-toxicity chemicals. Such alternatives to conventional fungicides for postharvest disease
100 control of fresh horticultural produce should be compounds with known and very low toxicological
101 effects on mammals and impact on the environment. According to their origin, these alternatives can
102 be roughly divided into synthetic and natural means. Among the former, the most important are
103 inorganic or organic salts, applied to fruit as aqueous solutions, and synthetic composite edible
104 coatings formulated with antifungal ingredients. The latter can be of plant or animal origin and
105 include plant extracts (including essential oils), antifungal peptides and small proteins, and natural
106 antifungal edible coatings such as chitosan and *Aloe* spp. gels. As food-grade substances, all

107 alternative chemicals should be approved as generally regarded as safe (GRAS) by the United States
108 Food and Drug Administration (US FDA), as food additives by the European Food Safety Authority
109 (EFSA), or as an equivalent status by each competent national legislation. GRAS materials are
110 exempt from residue tolerances on all agricultural commodities by the US FDA. In general, these
111 alternative chemicals can be applied to fresh fruit after harvest as aqueous solutions, vapors, or
112 coating treatments. Since essential oils and other antifungal gaseous compounds of plant origin are
113 primarily applied as fumigants, they are discussed with antifungal volatiles (Mari et al., 2016).

114 Due to their general low toxicity, application of these chemical alternatives by themselves
115 may not always provide a commercially acceptable level of control of postharvest diseases
116 comparable to that obtained with synthetic fungicides. For this reason, compounds with potential as
117 stand-alone treatments are increasingly being evaluated in combination with other postharvest
118 treatments of the same or different nature as part of ‘multiple hurdle’ or integrated control strategies.
119 Hurdle technology explores the use of mild treatments that collectively maintain the fruit quality and
120 lower the incidence of postharvest decay. In general, three different objectives may be pursued by
121 the combination of treatments (Palou et al., 2008): additive or synergistic effects to increase the
122 efficacy and/or the persistence of individual treatments, complementary effects to combine
123 preventive and curative modes of action, and commercial application of effective treatments that are
124 too impractical, costly, or risky as single treatments. Clear disadvantages of combined approaches
125 are their higher costs and complexity, which make greater the difficulty to turn their application in
126 commercial practice (Romanazzi et al., 2012). Integration of treatments highlighted in the present
127 chapter are the use of GRAS salts in combination with other alternative control means and combined
128 applications of chitosan, especially with essential oils as additional antifungal agents. Moreover,
129 from a strict point of view, composite edible coatings formulated with antifungal ingredients could
130 also be considered as combined treatments.

131

132 **2. Inorganic and organic salts**

133 Several inorganic and organic salts, classified as food additives or GRAS substances, have
134 been proved as more or less effective for the control of postharvest diseases of fresh horticultural
135 produce when applied as aqueous solutions after harvest. Most of these substances are listed as food
136 preservatives and have a well-known general antimicrobial activity. Although acidic forms also
137 possess antimicrobial activity in some cases, salts are preferred for postharvest treatments because of
138 their superior solubility, ease of application, and additional activity of cations such as Na⁺, K⁺, or
139 NH₄⁺ (Smilanick et al., 1999).

140 After being investigated in the early 30s of the last century in California (Barger, 1928), the
141 use of carbonate salts to treat citrus fruits was revisited in the 80s and 90s when problems related to
142 the continued use of conventional synthetic fungicides in citrus packinghouses started to arise. Dip
143 treatments in 2-3% sodium carbonate (SC) or sodium bicarbonate (SBC) aqueous solutions for 60-
144 150 s showed antifungal activity against citrus green and blue molds caused by *Penicillium digitatum*
145 and *Penicillium italicum*, respectively, and their performance was significantly improved by heating
146 the solutions to 45-50 °C. These treatments were not phytotoxic and also considerably reduced decay
147 on long-term cold stored fruit (Smilanick et al., 1997, 1999; Palou et al., 2001). In general, sodium
148 salts were more effective than other carbonate salts and their antifungal activity was higher on
149 oranges than on mandarins (Palou et al., 2002a). Since then, and after several successful commercial
150 applications attempted in California, SC and SBC have been the most common food preservatives
151 used for decay control in citrus packinghouses worldwide. Advantages are their relative
152 effectiveness, general low cost, and lack of restrictions for many applications including organic
153 agriculture. Many research works have focused on the evaluation of these salts, especially SBC, as
154 one of the components of integrated methods to control citrus postharvest diseases. Besides heating
155 the solutions, postharvest treatments that have been combined with carbonates for this purpose
156 include a number of antagonistic biological control agents, curing, UV-C light, different disinfectants
157 or oxidizers, and conventional fungicides such as imazalil (IMZ), pyrimethanil (PYR), or
158 thiabendazole (TBZ) at low doses (Palou et al., 2008; Dore et al., 2010; Venditti et al., 2010; Cerioni
159 et al., 2012, 2013; Geng et al., 2011; D'Aquino et al., 2013b; Hong et al., 2014). Carbonate salts
160 have also been applied before harvest or in combined applications before and after harvest (Youssef
161 et al., 2012). It was also in the decade of 1980 that the organic salt potassium sorbate (PS), a wide
162 spectrum antimicrobial food preservative, gained research attention for the control of citrus
163 postharvest diseases. Among others, extensive work by Kitagawa and Kawada (1984), Wild (1987),
164 Palou et al. (2002b), Smilanick et al. (2008), Montesinos-Herrero et al. (2009), and D'Aquino et al.
165 (2013a) showed that PS aqueous solutions at 2-3% were effective at different dip conditions, e.g. 2-3
166 min at room temperature or 30-60 s at 50-62 °C, against citrus green and blue molds and sour rot
167 caused by the yeast-like fungus *Geotrichum citri-aurantii*. Similarly to carbonates, the effectiveness
168 of these treatments was higher if applied at high temperature and it was clearly influenced by the
169 host species and cultivar, maturity stage, presence of peel wounds and fruit physical condition.
170 Likewise, they were compatible and also synergistic in some cases with low doses of chemical
171 fungicides such as IMZ, TBZ, PYR or fludioxonil (FLU). It was also recently showed that PS dips
172 followed by brief exposures to high CO₂ or O₂ at curing temperature were synergistic for the control
173 of green and blue molds (Montesinos-Herrero and Palou, 2016). PS-treated citrus fruits that will be

174 subjected to prolonged cold storage could be rinsed with tap water at low pressure without reducing
175 considerably the effectiveness of the treatment. It has been claimed that the presence of salt residues
176 on the rind could induce excessive water loss and adversely affect fruit quality (Parra et al., 2014).
177 Other GRAS salts with proven curative activity against citrus green and blue molds include sodium
178 benzoate (SB) (Montesinos-Herrero et al., 2016), sodium parabens (Moscoso-Ramírez et al., 2013),
179 and potassium silicate (PSi) (Moscoso-Ramírez and Palou, 2014). These authors established the best
180 concentration and treatment conditions for dip applications of aqueous solutions of these compounds.
181 All of them were compatible with the fungicide IMZ, their effectiveness was significantly higher on
182 oranges than on mandarins, and can be considered as new tools for potential inclusion in citrus
183 integrated disease management (IDM) programs. PSi, in addition, is included in the list of synthetic
184 substances allowed for use in organic crop production in the USA. A variety of salts have also been
185 evaluated for the control of citrus sour rot (Talibi et al., 2011; Duan et al., 2016).

186 The use of aqueous solutions of GRAS inorganic and organic salts as antifungal treatments to
187 control major postharvest diseases of stone fruits, i.e. peaches, nectarines, plums, and sweet cherries
188 has been recently reviewed (Usall et al., 2015). Several research works showed that postharvest
189 applications of SBC or potassium bicarbonate (PBC), alone or in combination with other alternative
190 control methods, effectively reduced brown rot caused by different species of *Monilinia* (mainly *M.*
191 *fructicola*, *M. laxa*, and *M. fructigena*). In other works, heated solutions of PS and SB were the most
192 effective treatments to reduce the incidence of brown rot, gray mold, blue mold, and sour rot, caused
193 by *M. fructicola*, *Botrytis cinerea*, *Penicillium expansum*, and *Geotrichum candidum*, respectively
194 (Palou et al., 2009; Molinu et al., 2012). On table grapes, reduction of the most economically
195 important postharvest disease, gray mold caused by *B. cinerea*, has been accomplished with
196 preharvest and/or postharvest treatments with salts such as SBC, SC, PS, PBC, potassium carbonate
197 (PC), or calcium chloride (CaCl₂) (Karabulut et al., 2005; Nigro et al., 2006; Youssef and Roberto,
198 2014). Due to their higher effectiveness and better integration into usual plant protection practices,
199 field applications, especially of CaCl₂, were recommended (Romanazzi et al., 2012). According to
200 common commercial handling in major table grape production areas, postharvest aqueous
201 applications of salts such as SBC or SC would be restricted to the detached berries industry (Mlikota
202 Gabler and Smilanick, 2001). Although not a salt, ethanol is also a GRAS compound with active
203 action against *B. cinerea* that could be of use when sulphur dioxide (SO₂) fumigations or grape
204 packaging with sodium metabisulphite pads are too risky or banned by particular markets
205 (Romanazzi et al., 2012). GRAS salts are also of application on table grapes in combination with
206 other decay control means. For instance, it was found in recent research that SBC enhanced gray
207 mold control and overall fruit quality on grapes treated with the biocontrol agent *Hanseniaspora*

208 *uvarum* (Qin et al., 2015). Similarly, SBC has been frequently evaluated as a component of
209 integrated treatments for the control of the most important postharvest diseases of pome fruits. Thus,
210 improved control of blue mold and black spot of apples or pears, caused by *P. expansum* and
211 *Alternaria alternata*, respectively, has been obtained by combining SBC application with a variety of
212 biocontrol agents, mainly yeasts, and other postharvest treatments such as heat or controlled
213 atmospheres (Yao et al., 2004; Janisiewicz et al., 2008; Janisiewicz and Conway, 2010; Lai et al.,
214 2015). Typically, SBC alone reduced decay in these trials, but was much more effective when
215 combined with the antagonists. In general, SBC or SC are good GRAS candidates for integration
216 with many biological control treatments thanks to the high compatibility that allow the proliferation
217 of microbial antagonists in fruit wounds containing carbonate residues. PS solutions have also been
218 recently tested against blue mold of apples alone or in combination with heat or TBZ (Fadda et al.,
219 2015). An active packaging comprised of polyethylene terephthalate coated with PS was recently
220 developed for gray mold control on raspberries, blackberries, and blueberries (Junqueira-Gonçalves
221 et al., 2016).

222 Inorganic and organic salts as postharvest antifungal treatments have been also applied to
223 tropical fruits such as avocado, mango, papaya or banana. Avocado production in countries like
224 South Africa, Israel or Chile is export driven, with the European Union (EU) being the biggest
225 market and this entails high fruit quality standards. Among them, the reduction or elimination of
226 chemical residues on/in fruit derived from fungicide use to control major postharvest diseases like
227 anthracnose caused by *Colletotrichum gloeosporioides* is increasingly gaining importance (van
228 Eeden and Korsten, 2013). Silicon (Si) has been reported as a beneficial nutrient, protecting plants
229 against various diseases. Anderson et al. (2005) reported on postharvest Si application to ‘Hass’
230 avocados intending to reduce occurrence of anthracnose. Postharvest PSi applications had no effect
231 on respiration rate, however fruit firmness, weight loss, mesocarp electrical conductivity (EC), total
232 phenolics concentration, lipid peroxidation as well as polyphenol oxidase (PPO) and catalase (CAT)
233 activity responded positively to the PSi treatments (Tsfay et al., 2011). These workers concluded
234 that silicon might function as a major elicitor increasing free polyphenol concentrations. Results
235 from recent work with mangos indicated that fruit treated with either bentonite or bentonite loaded
236 with PS exhibited reduced decay, delayed postharvest ripening, decreased water loss, maintained
237 high vitamin C levels, preserved titratable acidity (TA), and no changes in flavor (Liu et al., 2014).
238 Preharvest dips of mango fruit in plant defense inducing chemicals integrated with postharvest
239 treatments with inorganic salts and hot water were evaluated for the management of anthracnose on
240 artificially inoculated mango fruit. The application of either salicylic acid or potassium phosphonate
241 at 1000 mg L⁻¹ combined with a fruit dip for 3 min in 3% aqueous SBC at 51.5 °C significantly

242 reduced disease development as compared to other treatments and the control. The treatments also
243 maintained quality of mango, positively affecting pH, soluble solid content (SSC), TA, firmness, and
244 peel color. In contrast to SBC, CaCl₂ treatments alone or combined with preharvest plant defense
245 inducers did not significantly reduce anthracnose severity on mango (Dessalegn et al., 2013). The
246 effect of potassium tetraborate on germination of conidia of *C. gloeosporioides*, and postharvest rot
247 of mango were studied. An application of K₂B₄O₇ to mango trees at flowering increased fruit set and
248 decreased the incidence of anthracnose on harvested fruit (Shi et al., 2011). In the case of papaya,
249 anthracnose caused by *C. gloeosporioides* is also a major limiting factor in storage and transit that
250 affects all tropical regions where papaya is grown (Hewajulige and Wilson Wijeratnam, 2010). SBC
251 and ammonium carbonate (AC) in paraffin wax-based formulations have been shown to decrease
252 anthracnose in papaya (Gamagae et al., 2004). AC at 3% followed by SBC at 2%, tested alone or in
253 combination with wax, had a positive effect on reducing *C. gloeosporioides* in both naturally and
254 artificially inoculated fruit (Sivakumar et al., 2002). The effects of preharvest CaCl₂ application on
255 ripening, activity patterns of pectin modifying enzymes and overall quality of papaya fruit have been
256 investigated (Madani et al., 2014b). Foliar sprays of CaCl₂ at 0, 0.5, 1, 1.5, and 2% (w/v) were
257 applied six times during the growing season. The overall quality of treated fruit after 3 weeks at 12
258 °C was greater than that of the control. In a different work, Madani et al. (2014a) reported that
259 preharvest application of CaCl₂ at 1.5-2% increased calcium content in fruit and significantly
260 reduced anthracnose incidence during 5 weeks of storage at 12 °C, and delayed initiation of disease
261 symptoms by 4 weeks. Banana fruit are susceptible to several diseases resulting in massive and
262 extensive postharvest losses during transportation and storage. Crown rot, the most important
263 postharvest disease of banana, is a disease complex caused by several fungi including *Lasiodiplodia*
264 *theobromae*, *Colletotrichum musae*, *Thielaviopsis paradoxa*, and a complex of *Fusarium* spp.
265 (Ranasinghe et al., 2005). Postharvest application of inorganic salts has been evaluated as an
266 alternative to fungicide dips used for commercial management of banana crown rot. Dipping bananas
267 for 10-15 min in SBC, CaCl₂, sodium chloride (NaCl), or sodium hypochlorite (NaClO) solutions
268 significantly reduced the incidence of crown rot compared with untreated fruit 17 days after harvest.
269 SC solutions were ineffective (Alvandia et al., 2004). In other work, effective treatments to control
270 crown rot of bananas were SBC and NaClO at 5 g L⁻¹ and CaCl₂ at 5 g L⁻¹ with a surfactant. Some
271 salts, when ameliorated with surfactant, had a phytotoxic effect on banana fruit (Alvandia and
272 Natsuaki, 2007). Natural infections of anthracnose, crown rot, and blossom-end rot were reduced
273 significantly in banana fruit that were treated with 300 mM SBC for 10 min. This treatment followed
274 by dips in a suspension of the bacterial antagonist *Burkholderia spinosa* was also effective. SBC dips
275 increased pH, SSC and thickness of the fruit peel, which could have an indirect or cumulative effect

276 on the reduction of postharvest disease development in bananas (De Costa and Gunawardhana,
277 2012). The efficacy of SC, SBC, and NaClO, applied alone or in various combinations with another
278 antagonist, *Bacillus amyloliquefaciens* DGA14, was evaluated. The results indicated that the
279 combination of *B. amyloliquefaciens* with 1% SBC managed crown rot disease comparable with
280 synthetic fungicides without negative effects on fruit quality 14 days after treatment (Alvandia,
281 2013b). In a different approach, salts (SBC, SC, NaClO) were integrated with a hot water treatment
282 (HWT, 50 °C for 20 min). Postharvest application, involving fruit dipped for 30 min in 1% SC
283 following HWT, and storage for 14 days at 22-25 °C and 90-95% relative humidity (RH), maintained
284 the overall quality of bananas and reduced the incidence of crown rot disease by 88%, which was
285 comparable with the efficacy of a conventional fungicide treatment. Other treatment combinations
286 such as HWT + NaClO or HWT + SBC were also capable of reducing crown rot, although to a lesser
287 extent. Efficacy of individual treatments, and particularly of HWT alone, was significantly lower
288 (Alvandia, 2013a). In other research, a combination of the fungal biocontrol agent *Trichoderma*
289 *harzianum* and 1% SBC was the best for crown rot control, with an efficacy similar to synthetic
290 fungicides, and maintained the overall quality of banana even at conditions favorable for the
291 pathogens (22-25 °C, 90-95% RH) (Alvandia, 2013c).

292 Some inorganic and organic salts have also been evaluated as antifungal treatments against
293 important postharvest pathogens of vegetables or fruit-like vegetables such as tomato or potato.
294 CaCl₂ was tested for the control of postharvest decay development on cherry tomatoes alone or in
295 combination with the marine yeast *Rhodospiridium paludigenum*. It was verified that the combined
296 treatments showed high activity to reduce black rot caused by *A. alternata* (Wang et al., 2010). The
297 inhibitory effect of CaCl₂ alone or in combination with cassia oil against this disease was also
298 assessed on cherry tomato. Some combinations showed a significant inhibition effect on decay
299 development in both artificially wounded and unwounded naturally infected fruit. Importantly, these
300 treatments did not reduce the overall quality of tomato. It was observed that the treatments
301 significantly enhanced the activity of some defense-related enzymes such as PPO and peroxidase
302 (POD) (Feng et al., 2013). Mature red cherry tomato fruit were treated with HWT (45 °C) or 2%
303 SBC, each alone or in combination for 10 min, then stored at 20 °C for 6 days. HWT alone was not
304 suitable for mature red cherry tomato fruit, while a combination of HWT plus SBC showed potential
305 as a commercial treatment to avoid fruit cracking, improve safety, and maintain fruit quality.
306 Compared with the individual treatments, the combined treatment also reduced the residual
307 procymidone fungicide content of the fruit. After storage, fruit subjected to the combined treatment
308 had higher skin firmness and TA, with a lower incidence of gray mold caused by *B. cinerea* (Shao et
309 al., 2012). On potato, in vitro trials were conducted to evaluate the effect of several organic and

310 inorganic salt compounds at three different concentrations on the development of postharvest
311 pathogens such as *A. alternata*, *B. cinerea*, *Fusarium solani* var. *coeruleum*, *Phytophthora* spp., and
312 *Verticillium* spp. Overall, mycelium growth and spore germination of all pathogens were strongly
313 inhibited by sodium metabisulfite and propyl-paraben. Spore germination in most pathogens was
314 consistently inhibited by various aluminum salts (Mills et al., 2004). In other research, several salts
315 were found to control bacteria such as *Pectobacterium carotovorum* subsp. *carotovorum* (formerly
316 *Erwinia carotovora* subsp. *carotovora*) and *Pectobacterium atrosepticum* (formerly *Erwinia*
317 *carotovora* subsp. *atroseptica*), the causal agents of blackleg and tuber soft rot, respectively, that can
318 contaminate potato tubers both in the field and after harvest. Potato tubers treated with SBC, NaClO,
319 aluminum acetate, alum, calcium propionate, or copper sulfate pentahydrate resulted in significantly
320 less soft rot than the untreated control. These compounds demonstrated a potential as replacements
321 for commercially used postharvest pesticides (Mills et al., 2006). In recent work, soft rot of potato
322 tubers was significantly controlled with aluminum chloride (AlCl₃) and sodium metabisulfite, and to
323 a lesser extent with SB, PS, SP, and aluminum lactate (Yaganza et al., 2014).

324 Although the exact mode of action of many GRAS salts on reducing postharvest disease has
325 not been completely explained, there is evidence that their inhibitory ability depends on the presence
326 of salt residues in the infection courts occupied by the pathogen, typically fruit peel wounds, and on
327 interactions between this residue and constituents of the fruit tissues. Among such interactions,
328 particular direct toxic action of the different anions and cations, pH alterations, and indirect factors
329 related to the induction of disease resistance mechanisms on the fruit host (lignification, biosynthesis
330 and/or accumulation of antifungal compounds correlated with the up-regulation of the
331 phenylpropanoid pathway, etc.) have been described (Palou et al., 2001; Venditti et al., 2005;
332 Youssef et al., 2014). The fact that the induction of resistance is dependent on host factors like the
333 genotype (species and cultivar), maturity stage, and physical and physiological condition can explain
334 why these factors also strongly influence the effectiveness of salt treatments (Moscoso-Ramírez et
335 al., 2013). This dependence, the lack of residual effect to protect the fruit against subsequent
336 infections, the risks of adverse effects on fruit quality during long-term storage, and, above all, the
337 lower efficacy and persistence of the treatments are general disadvantages of GRAS salt treatments
338 compared with conventional fungicides. Additional problems are the lack of regulatory approval for
339 many compounds evaluated in the laboratory and disposal issues related to high salinity, sodium
340 content, pH, and conductivity of some salt aqueous solutions (Palou et al., 2008; Smilanick et al.,
341 2008). Besides the residue tolerance that makes them attractive as control alternatives, in some cases
342 even for organic agriculture, general advantages are their significant curative effect, availability at

343 low cost, and especially the high complementarity with other treatments that would allow their use as
344 hurdle technologies in postharvest IDM programs.

345

346 **3. Plant extracts**

347 Essential oils are the most important compounds among the wide range of natural substances
348 with antifungal properties extracted from plants. They comprise of a combination of volatile
349 secondary metabolites that are bioactive in vapor phase, show direct activity against phytopathogens,
350 and can also enhance the plant defense mechanisms against these microorganisms (Bautista-Baños et
351 al., 2013). Some of the key active ingredients in essential oils, e.g. cinnamaldehyde, citral, eugenol,
352 limonene, or thymol, are safe for human consumption and have a GRAS status (de Aquino et al.,
353 2015). Therefore, they have been extensively investigated for postharvest applications. Since they
354 have been mainly applied in the vapor phase, they are covered together with antifungal volatile
355 compounds (Mari et al., 2016). However, a viable option to overcome important limitations
356 associated with the use of essentials oils, namely induction of strong odors or flavors, phytotoxicity
357 risks, and lack of efficacy in vivo, would be the incorporation of essential oils into edible coatings
358 and this technology will be discussed later in this review.

359 In this section, we refer to other aqueous or organic solvent extracts of plants or herbs that
360 have been reported as able to reduce decay on harvested horticultural products. Many of these
361 extracts have been obtained from medicinal or exotic plants located in African, Asian, or South
362 American countries. In general, they are biodegradable substances comprised of compounds that
363 exhibit direct fungicidal or fungistatic activity and also some ability to delay ripening and extend the
364 shelf life of treated produce. Typically, these compounds are products of secondary metabolism
365 produced by the plant for its own protection against pests and pathogens. Extracts from plants that
366 presented a significant activity against the most important citrus postharvest pathogens *P. digitatum*,
367 *P. italicum*, or *G. citri-aurantii* include garlic (Obagwu and Korsten, 2003), pomegranate (Li Destri
368 Nicosia et al., 2016), *Sanguisorba* sp., *Orobanch* sp. (Gatto et al., 2011), Huamuchil (Barrera-
369 Necha et al., 2003), *Accacia* sp., *Whitania* sp. (Mekbib et al., 2009), and *Parastrephia* sp. (Ruíz et
370 al., 2016), among many others (Palou et al., 2008; Askarne et al., 2012; Sayago et al., 2012; Talibi et
371 al., 2012). Some of these extracts have also been found to present antifungal activity against
372 pathogens attacking other fresh produce. This is the case of garlic extracts on apples (Daniel et al.,
373 2015) and bananas (Sanwal and Payasi, 2007), or pomegranate extracts on sweet cherries (Li Destri
374 Nicosia et al., 2016), table grapes (Romeo et al., 2015), and potato tubers (Elsherbiny et al., 2016). In
375 the case of stone fruits, plant extracts with activity against *Monilinia* spp. and other postharvest
376 pathogens have been recently listed in a review by Usall et al. (2015). Some extracts from seed

377 kernel of neem plant (*Azadirachta indica*) have been shown to be toxic to various fungal pathogens
378 causing postharvest decay on plums and pears (Wang et al., 2010). A commercial formulation based
379 on extracts from *Abies* spp. was able to control postharvest diseases of strawberries and sweet
380 cherries when applied in the field (Feliziani et al., 2013a; Romanazzi et al., 2013). However, as most
381 of treatments applied on fruit and vegetables, it is important to check the sensorial quality of treated
382 produce, which should be better or at least not change as compared to the common practice. With
383 respect to tropical fruits, plant extracts from the botanical families Sapotaceae (*Achras sapota*,
384 *Chrysophyllum cainito* and *Pouteria sapota*), Caricaceae (*C. papaya*), Fabaceae (*Pachyrrizus*
385 *erosus*), Leguminosae (*Phytechellobium dulce*), Solanaceae (*Cestrum nocturnum*), and Verbenaceae
386 (*Lantana camara*) showed noteworthy control of various fungal diseases of papaya such as those
387 caused by *C. gloeosporioides*, *Rhizopus* spp., *Aspergillus* spp. and *Mucor* spp. (Bautista-Baños et al.,
388 2013). Likewise, the constituents and secondary metabolites of *Pachyrrizus dulce* and *P. erosus*
389 demonstrated a remarkable control of various postharvest fungi of papaya (Barrera-Necha et al.,
390 2004). Celoto et al. (2011) evaluated the use of methanol and aqueous extracts of *Momordica*
391 *charantia* applied on banana fruit, observing up to 80% inhibition in the development of lesions
392 caused by *C. musae* when applied 2 days prior to fungus inoculation. In another research with
393 bananas, artificially inoculated fruit dipped in 20% (w/v) extracts of *Acacia albida* and *Prosopis*
394 *juliflora* at 50 °C showed reduced anthracnose incidence and severity. This combined treatment did
395 not affect the fruit physico-chemical properties and provided the highest percentage of marketable
396 ripe banana fruit (Bazie et al., 2014). Black rot of pineapple caused by the fungus *Chalara paradoxa*
397 was considerably reduced by the application of an extract of *Mormodica charantia* without affecting
398 the postharvest quality of treated fruit (de Souza et al., 2015). Among various plant species tested,
399 aqueous extracts of leaves of papaya and custard apple (*Annona reticulata*) showed important
400 fungistatic effects against *Rhizopus stolonifer* and *C. gloeosporioides* on mango and ciruela
401 (*Spondias purpurea*) during fruit storage (Bautista-Baños et al., 2000, 2003).

402 Isothiocyanates (ITCs) are extracted from several families of plants that include Brassicaceae,
403 Moringaceae and Resedeaceae (Bautista-Baños et al., 2013). These naturally occurring compounds
404 are the degradation products of glucosinolates and are toxic to many organisms including fungi,
405 bacteria, nematodes, insects and weeds. Their general use against fungal diseases was reviewed by
406 Tiznado-Hernández and Troncoso-Rojas (2006). As antifungal postharvest treatments, they were
407 tested in vivo against pathogens of pome fruits, stone fruits, and strawberries such as *B. cinerea*, *R.*
408 *stolonifer*, *Mucor piriformis*, *P. expansum*, and *M. laxa* (Mari et al., 1996, 2008; Ugolini et al.,
409 2014), and a method based on the use of allyl-isothiocyanate on citrus fruit was patented in Japan
410 (MITN-C, 2004). They were also applied for inhibition of the growth of *C. gloeosporioides*,

411 *Fusarium oxysporum* and *R. stolonifer*, and for controlling fungal infection of papaya (Ramos-García
412 et al., 2007). Application of phenylethyl ITC was demonstrated to be more effective than the
413 fungicide benomyl. Moreover, Troncoso-Rojas et al. (2005) reported satisfactory control of *A.*
414 *alternata* in vitro and alternaria rot on bell pepper using 0.03 mg mL⁻¹ ITC extracted from cabbage
415 leaves. In another work, benzyl ITC was also effective against alternaria rot on tomato (Troncoso-
416 Rojas et al., 2005). ITCs are natural occurring volatile compounds with a broad spectrum of activity
417 and are safe for human consumption, thus are suitable alternatives for synthetic fungicides.

418 Propolis is a natural compound made up of esters, carbohydrates, diterpenic acids, and
419 pentacyclic triterpenes. It is used by bees for protecting their hives (Kasote et al., 2015). It is sourced
420 from conifer trees and is characterized with potent antimicrobial and antibiotic activity. Soylyu et al.
421 (2008) observed that 70 and 35% ethanol extracts of propolis completely inhibited conidia
422 germination of the citrus postharvest pathogen *P. digitatum*, but failed to control green mold on
423 artificially inoculated grapefruits. Similarly, ethanol extracts of Chinese propolis completely
424 prevented the in vitro mycelial growth of *P. italicum* (Yang et al., 2011). However, no in vivo tests
425 for evaluation of blue mold control were conducted by these workers. Mattiuz et al. (2015)
426 demonstrated the efficacy of propolis in controlling the growth of *C. gloeosporioides* in vitro and
427 reducing anthracnose on mango in vivo. In a study by Ordones et al. (2011), antimicrobial activity of
428 propolis against *Erwinia carotovora* spp. *carotovora*, *Pseudomonas syringae* pv. *tomato*,
429 *Pseudomonas corrugata* and *Xanthomonas campestris* pv. *vesicatoria* was demonstrated at
430 concentrations as low as 9.5 µg mL⁻¹. The authors also demonstrated the activity in controlling *P.*
431 *syringae* infection of tomato. A major concern with the application of propolis, as with volatile
432 compounds, is the possibility of the treatment causing phytotoxicity on the surface of the fruit in
433 addition to the effect on flavor and aroma if applied at high doses.

434

435 **4. Antifungal peptides and small proteins**

436 Peptides produced by plants or animals as a defense mechanism against challenging microbes
437 are known, in general, as antimicrobial peptides (AMPs). In parallel to what has occurred in
438 medicine, AMPs and small proteins have been proposed for potential use in agriculture as novel
439 therapeutics for the control of plant diseases. However, limitations of natural peptides such as
440 nonspecific toxicity, low stability, and poor bioavailability led researchers to attempts to artificially
441 synthesize new AMPs with superior properties (Marcos et al., 2008). In this case, production and
442 purification costs are important limitations and alternatives like the use of plants as biofactories
443 through genetic engineering techniques are being increasingly studied (Bundó et al., 2014).
444 Antifungal peptides and proteins are typically short compounds of amphipathic cationic nature,

445 which mechanism of action is presumably the disruption of the target fungal cell membrane. For the
446 control of postharvest diseases of horticultural produce, the production of antifungal peptides (e.g.
447 iturins, fengycins, etc.) has been frequently identified as a mode of action of several biological
448 control agents, mainly bacteria, e.g. *Bacillus* spp. (Yáñez-Mendizábal et al., 2012; Waewthongrak et
449 al., 2015). Moreover, some plant-produced peptides have shown activity against postharvest
450 pathogens (Alem et al., 2014), and non-natural compounds have been synthesized that also showed
451 promise as effective postharvest treatments. This is the case of the tryptophan-rich, cationic
452 hexapeptide PAF26 and some derivatives, which showed inhibitory activity against citrus pathogens
453 like *P. digitatum* (Muñoz et al., 2007; Harries et al., 2015), or the hybrid undecapeptide BP22, which
454 resulted active against *P. expansum*, the cause of blue mold on pome and stone fruits (Badosa et al.,
455 2009).

456

457 **5. Antifungal edible coatings**

458 Artificial coating of fresh horticultural produce is common in many packinghouses to reduce
459 weight loss, shrinkage, and improve appearance. Typically, commercial coatings are wax-based
460 compounds, often amended with synthetic fungicides to additionally provide control of postharvest
461 diseases. Currently, there is an increasing interest in the development of antifungal edible coatings of
462 natural (plant or animal) origin or based on biodegradable formulations amended with additional
463 food-grade antifungal compounds in order to replace these commercial waxes. While chitosan-based
464 or *Aloe vera*-based coatings present inherent antimicrobial activity, synthetic biopolymer-based
465 coatings are formulated with antimicrobial ingredients that, according to their nature, can belong to
466 three different categories (Palou et al., 2015): i) synthetic food preservatives or GRAS compounds
467 such as various inorganic and organic salts, ii) natural compounds such as essential oils or other
468 natural plant extracts, and iii) microbial antagonists as biocontrol agents (bacteria, yeast, yeast-like
469 fungi, and even some filamentous fungi, covered in the review by Droby et al. (2016)). As
470 components of edible formulations, compounds in the first two groups should be classified as food-
471 grade additives or GRAS compounds by the regulation agencies.

472

473 *5.1. Chitosan and derivatives*

474 Chitosan is a natural, biodegradable, biocompatible, and non-toxic biopolymer obtained from
475 chitin deacetylation that is sourced from the exo-skeleton of crustaceans (Muzzarelli and Muzzarelli,
476 2005; Lizardi-Mendoza et al., 2016). It is a biopolymer that needs to be dissolved in weak acids, in a
477 solution having a pH of at least 2.8 (usually acetic acid at 0.5 or 1%), and its antimicrobial activity
478 and physical properties (e.g. viscosity, coating thickness) varies according to the acid used for

479 dissolution (Romanazzi et al., 2009). There is a high interest for its use in agriculture because when it
480 is applied on plants, it shows a triple action: antimicrobial activity on plant pathogens, film-forming
481 activity that acts as barrier, and ability to elicit plant defense mechanisms, that makes it an ideal
482 coating for fruit and vegetables (Romanazzi et al., 2016). Chitosan possess direct antimicrobial
483 properties and has been proven to control fungi and bacteria in vitro and in vivo, including *C.*
484 *gloeosporioides*, *R. stolonifer*, *P. digitatum*, and *F. oxysporum* (Bautista-Baños et al., 2013). Growth
485 inhibition induced by chitosan on *P. digitatum*, *P. italicum*, *Botryodiplodia lecanidion*, and *B.*
486 *cinerea* ranged from 25 to 95% (Chien and Chou, 2006). At 1%, it decreased the radial growth of
487 decay causing fungi like *B. cinerea*, *A. alternata*, *M. laxa* and *R. stolonifer* at the same rate or close
488 to the fungicide fenhexamide (Feliziani et al., 2013a). Moreover, it was found effective in the control
489 of a list of foodborne bacteria, mainly when used as chitosan nanoparticles (Sotelo-Boyás et al.,
490 2016). On the other hand, chitosan produces a film on the treated fruit surface that reduce gas
491 exchange and respiration (El Ghaouth et al., 1991; Romanazzi et al., 2009), then slowing down
492 ripening and keeping the fruit less susceptible to decay. Chitosan hydrochloride was approved in the
493 EU as the first product in the list of basic substances in plant disease management (Reg. EU
494 563/2004) and some commercial formulations should soon be registered for use as plant protection
495 products. This aspect will increase its use and lower the cost of formulations, so it will be easier for
496 growers to test the feasibility of large scale chitosan applications, both in organic and in conventional
497 agriculture, to improve the management of postharvest decay.

498 Chitosan and derivatives are currently the most assayed antifungal edible coatings for
499 postharvest preservation of fresh horticultural produce. Chitosan has been applied to prolong storage
500 and shelf life of a long list of temperate fruit, including apple, pear, peach, sweet cherry, strawberry,
501 blueberry, raspberry, and table grapes, among others (Romanazzi et al., 2016). Thirty years ago,
502 Muzzarelli (1986) reported that chitosan had all properties of an ideal biopolymer for coating of
503 fruits and vegetables. The research on the application of chitosan on fruit was started in the last
504 decade of the last century by Ahmed El Ghaouth and coworkers (El Ghaouth et al., 1991, 1992a) in
505 Joseph Arul's laboratory at the University of Laval (Canada). They used chitosan on strawberry fruit
506 kept at 13 °C, finding an extended storage and a reduction of gray mold and Rhizopus rot, with a
507 concurrent direct effect (radial growth inhibition and hyphal deformation) on *B. cinerea* and *R.*
508 *stolonifer*. In the meantime, they applied chitosan to protect tomatoes, which appears to be the first
509 application on vegetables (El Ghaouth et al., 1992b). Since then, several research groups found
510 interest in the use of chitosan, and further information on its coating properties were observed on
511 strawberries (Zhang and Quantick, 1998; Reddy et al., 2000b), table grapes (Romanazzi et al., 2002;
512 Meng et al., 2008), and sweet cherries (Romanazzi et al., 2003) sprayed in the field with chitosan

513 formulations. Most of the first trials were run with chitosan powder dissolved in weak acids, and in
514 the last years a list of commercial formulations were made available on the market (Elmer and
515 Reglinski, 2006; Romanazzi et al., 2016). Some commercial chitosan formulations had the same
516 effectiveness than practical grade chitosan dissolved in weak acids (Feliziani et al., 2013b, 2015).
517 However, water dissolvable chitosan formulations are much easier to use for growers and have a
518 higher potential interest. One of the most important aspects researchers focused on was the effect of
519 chitosan applications on fruit quality. In trials run on strawberries, chitosan treatments did not affect
520 the taste of the fruit after application, except very soon after application, when a slightly bitter taste
521 was perceived (Devlieghere et al., 2004). In general, in most of the studies, overall fruit quality was
522 not negatively affected by chitosan application (El Ghaouth et al., 1991; Devlieghere et al., 2004;
523 Feliziani et al., 2015). Another positive aspect of chitosan versus synthetic fungicides is its typical
524 persistence on plant tissues. In trials in which it was applied once, 21 days before harvest, or twice,
525 21 and 5 days before harvest, no differences in decay development on table grapes were observed
526 after 30 days of cold storage and 4 days of shelf life (Romanazzi et al., 2002).

527 Chitosan has also been evaluated on a variety of citrus and other subtropical and tropical
528 fruits. Significant reductions of citrus green or blue molds were obtained in laboratory trials with
529 oranges, lemons, mandarins, or grapefruits artificially inoculated with *P. digitatum* or *P. italicum* and
530 treated with chitosan or derivatives such as glycol chitosan (El Ghaouth et al., 2000; Chien et al.,
531 2007; Zeng et al., 2010; Panebianco et al., 2014). Both direct and indirect effects (enhanced activity
532 of enzymes such as POD or superoxide dismutase (SOD)) for disease reduction were reported in
533 these studies. Furthermore, oligochitosan, another hydrolyzed derivative, was effective to reduce
534 anthracnose caused by *C. gloeosporioides* on oranges (Deng et al., 2015). On papaya fruit treated
535 with chitosan and stored at room temperature, 150 kDa chitosan successfully controlled mesophilic
536 bacteria, yeast, and molds, extending the shelf life of the fruit for 4 to 7 days (Dotto et al., 2015).
537 Hong et al. (2012) reported that application of 2% chitosan coating on ‘pearl’ guava stored at 11 °C
538 for 12 days significantly enhanced the antioxidant ability of the fruit, which significantly delayed
539 fruit ripening.

540 While studies on the application of chitosan on fruit is popular, at least at research stage, the
541 use of chitosan to manage postharvest decay of vegetables is not deeply studied as well. After first
542 investigations by El Ghaouth et al. (1992b) on tomatoes, it was later applied on the same crop with
543 positive results in terms of decay management and fruit quality maintenance (Reddy et al., 2000a;
544 Liu et al., 2007; Badawy and Rabea, 2009). The application of chitosan on vegetables as asparagus,
545 broccoli, carrot, green beans, potato, radish, red bell pepper, squash, and sweet pepper was recently
546 reviewed by Miranda-Castro (2016).

547 Nanotechnology applied for development of innovative chitosan-based coatings has been a
548 new direction explored in recent years. Chitosan nanoformulations are characterized by the smaller
549 size, which allows for encapsulation of the functional ingredients and reduced chemical degradation
550 (Mustafa et al., 2013). Ing et al. (2012) demonstrated the antimicrobial activity of chitosan in vitro
551 against the pathogens *Fusarium solani* and *Aspergillus niger*, which was more effective when the
552 high molecular weight chitosan was used to prepare the nanoformulation. Applications of chitosan
553 nanoformulations at 1, 1.5, or 2% were similarly effective in controlling the growth of
554 *Colletotrichum musae* and *C. gloeosporioides* (Zahid et al., 2012). The authors also demonstrated the
555 effectiveness of the chitosan nanoformulation at concentration of 1% in controlling anthracnose of
556 banana, papaya, and dragonfruit. The use of nanoformulation allowed the authors to apply lower
557 doses of chitosan (1%) than would normally be applied (1.5 or 2%). Nanotechnology was also
558 explored by Jiang et al. (2013) in the application of alginate/nano-Ag coatings for reducing the
559 microbial load and extending the shelf life of shiitake mushrooms.

560

561 5.2. Combined applications of chitosan

562 Chitosan has the properties to be applied alone, but it works well when combined with other
563 alternatives to synthetic fungicides in order to exploit additive or synergistic effects. Although
564 chitosan alone shows antimicrobial activity at standard doses (usually 1%), reducing the rates is
565 compatible with the application of other disease control methods of different nature such as inorganic
566 and organic salts, ethanol, UV-C irradiation, plant extracts, essential oils, biocontrol agents, modified
567 active packaging, or hypobaric treatment. Further, chitosan is also compatible with the addition of
568 vitamins, ascorbic acid, or other bioactive compounds that can enhance fruit quality (Romanazzi et
569 al., 2012, 2016).

570 Early work by El Ghaouth et al. (2000) showed that glycol chitosan, a water dissolvable
571 chemical formulation of chitosan, combined with SC and a biocontrol yeast improved the control of
572 postharvest decay of apple and citrus fruit. Sivakumar et al. (2005) reported that chitosan alone or in
573 combination with SBC or AC significantly reduced the severity of anthracnose in papaya fruit. The
574 effect of chitosan with AC on the incidence and severity of anthracnose was greater than chitosan
575 alone, or chitosan with SBC. Eating quality was not affected by these postharvest dip treatments. A
576 combination of chitosan with AC retained high fruit quality, significantly retarded color development
577 of skin and flesh, increased fruit firmness and reduced weight loss. Al Eryani-Raqeeb et al. (2009)
578 demonstrated the efficacy of calcium (2.5%) and chitosan (0.75%) infiltration in controlling *C.*
579 *gloeosporioides*. The development of this fungus on fruit treated with this combination was seriously
580 affected since no conidial germination took place and the incidence of anthracnose was 38%

581 compared with the 88% shown in the untreated fruit. Additional advantages of this treatment were an
582 extended storage life of approximately 15 days and reduced weight and firmness loss.

583 There is no doubt the most frequent combined application of chitosan is the incorporation of
584 plant extracts, essential oils or their components into chitosan coating matrixes. In general, this
585 allows a unique postharvest treatment with additional properties for fungal growth inhibition and
586 fruit quality maintenance (Palou et al., 2015). Chitosan combined with citral or lemongrass oil
587 significantly controlled green and blue molds and sour rot on artificially inoculated oranges and
588 limes (Faten, 2010; El-Mohamedy et al., 2015). Chitosan coatings amended with essential oils from
589 bergamot, thyme, or tea tree were more effective than chitosan alone in reducing blue mold of
590 oranges caused by *P. italicum*, providing both preventive and curative activity (Cháfer et al., 2012).
591 It was concluded in another work that chitosan improved the release of Mexican oregano, cinnamon,
592 or lemongrass essential oils to inhibit the pathogens *P. digitatum* and *A. niger* (Avila-Sosa et al.,
593 2012). Nevertheless, in a recent study by Shao et al. (2015) the combination of chitosan with clove
594 oil for inhibiting *P. digitatum* on citrus fruit did not demonstrate enhanced activity in comparison
595 with treatment with chitosan alone, despite that the combination of chitosan and clove oil enhanced
596 the activity of some fruit defense enzymes. Working with guava fruit, de Aquino et al. (2015)
597 explored the activity of chitosan-cassava starch coating in combination with the essential oils
598 extracted from *Lippia gracilis*, which contained mostly thymol and carvacrol. Combination of the
599 three constituents at 2% chitosan, 2% cassava and 1, 2 and 3% essential oil were effective in
600 controlling microbial infection of the fruit. Mattiuz et al. (2015) reported that the performance of
601 propolis on mango in vivo for the control of anthracnose and maintenance of fruit quality was better
602 for fruit treated with 1.5% chitosan. Barrera et al. (2015) explored propolis at 5% in combination
603 with 1% chitosan for controlling anthracnose of papaya and reported reduced fruit decay and
604 infection diameter of the causal agent of anthracnose in papaya, *C. gloeosporioides*. Bautista-Baños
605 et al. (2003) found that the combination of 2.5% chitosan with aqueous extracts of custard apple
606 leaves, papaya leaves, or papaya seeds had a fungistatic rather than fungicidal effect on the
607 development of *C. gloeosporioides*. Chitosan applications did not influence SSC or weight loss
608 during the storage of papaya fruit. However, there was a tendency toward greater firmness in fruit
609 treated with the papaya seed extract alone or combined with chitosan. On vegetables, the combined
610 application of chitosan and cinnamon oil improved decay control of sweet pepper (Xing et al., 2011),
611 while when it was combined with natamycin, it reduced *Fusarium* and *Alternaria* rots of melon
612 (Cong et al., 2007). In general, the activity of essential oils is attributed to the presence of active
613 ingredients, characterized with both antimicrobial and antioxidant properties (Sivakumar and
614 Bautista-Baños, 2014). The activity of chitosan plus essential oils operates either through direct

615 effect on the pathogen or through enhanced plant defense mechanisms such as phenylalanine
616 ammonia-lyase (PAL), chitinase (CHI), and β -1,3 glucanase activity (Zhang et al., 2011).

617 Chitosan has been recently explored in combination with plant growth regulators for
618 enhanced control of fruit shelf life. A chitosan-g-salicylic acid complex was found to maintain the
619 quality attributes of cucumber, while inhibiting chilling injury (Zhang et al., 2015). Moreover,
620 antioxidant activity of the fruit (SOD, APX and CAT), as well as endogenous salicylic acid content,
621 was enhanced by this treatment, which directly enhanced the defense mechanisms and quality
622 attributes of the vegetable. Kumari et al. (2015) also explored the combination of chitosan and
623 salicylic acid on litchi fruit stored at 4 °C. The treatment was found to delay decay loss of the fruit,
624 while maintaining the fruit quality and nutraceutical content, reducing susceptibility to disease.
625 Qiuping and Wenshui (2007) treated Indian jujube fruit with the ethylene inhibitor 1-
626 methylcyclopropene (1-MCP) and then coated the fruit with chitosan. The combination of the
627 treatments was effective in extending the shelf life of the fruit by a total of 8 days and enhancing the
628 bioactive constituents such as ascorbic acid content, which are important in natural defense
629 mechanisms.

630 While chitosan is a strong antifungal agent, it can block fruit respiration when applied at high
631 concentrations. A novel application of chitosan is the development of bilayer coatings, which have
632 the capacity to provide a uniform matrix that delivers the desirable properties of chitosan without
633 deteriorating the fruit quality. Arnon et al. (2014) applied a bilayer coating comprising of chitosan
634 and carboxymethyl cellulose (CMC) as a treatment for citrus fruit. They reported enhanced firmness
635 retention, which would greatly reduce the susceptibility of the fruit to infection by phytopathogens.
636 Mustafa et al. (2013) and Ali et al. (2014) reported a better delivery of chitosan when applied as
637 nanoformulation into the fruit, but they also observed poorer permeability properties for chitosan.
638 Thus, Ali et al. (2014) explored the benefits of bilayer coatings by coating fruits with a layer of 600
639 nm chitosan nanoemulsion, followed by a layer of 1% conventional chitosan coating for maintaining
640 the quality of dragon fruit during storage for 28 days.

641

642 5.3. *Aloe spp.* coatings

643 Gels and aqueous extracts from the leaves of the plant *Aloe vera*, but also from other *Aloe*
644 spp. such as *A. arborescens* or *A. ferox*, show well-known bioactive and antimicrobial activity and
645 have been used as raw materials for many uses in different industries (medicinal, pharmaceutical,
646 cosmetic, tonic drinks, and others in the food industry), including the treatment of horticultural
647 products. The gels have a complex chemical composition, mainly polysaccharides and soluble
648 sugars, followed by proteins, many of which are enzymes, aminoacids, vitamins, and anthraquinones

649 (Zapata et al., 2013). Similarly to chitosan, the physical and chemical properties of the gels allow
650 their use as edible coatings for physiologic preservation of fruits and vegetables, but they have been
651 less studied as antifungal treatments. Early work by Saks and Barkai-Golan (1995) showed
652 significant activity of *A. vera* gels against *P. digitatum* and against green mold on grapefruits
653 artificially inoculated with this fungus. Recently, Jhalegar et al. (2014) found that an *A. vera*-based
654 coating reduced green and blue molds on mandarins. Different research showed the in vitro
655 inhibitory activity of *A. vera* gels against the most important postharvest pathogens of stone fruits
656 and table grapes, and also the high antifungal action of preharvest or postharvest coating applications
657 of these gels on peaches, plums (Guillén et al., 2013), nectarines (Navarro et al., 2011), sweet
658 cherries (Martínez-Romero et al., 2006), table grapes (Valverde et al., 2005; Castillo et al., 2010),
659 strawberries (Sogvar et al., 2016), or avocados (Bill et al., 2014). Most of these studies also
660 demonstrated the good performance of these coatings to delay ripening and preserve functional
661 properties and overall quality of treated fruit. In addition, the activity of *A. vera* coatings has been
662 reinforced in some cases through the incorporation of additional ingredients like essential oils or
663 acetic acid (Bill et al., 2014; Paladines et al., 2014; Sogvar et al., 2016).

664

665 5.4. Composite antifungal edible coatings

666 Substantial research has been devoted in recent years to the development of novel synthetic,
667 food-grade composite coatings with antimicrobial properties. The term composite indicates that the
668 matrix of the coating contains a combination of hydrocolloids (polysaccharides or proteins) with
669 lipids (waxes, acylglycerols, or fatty acids). In general, hydrocolloids provide good gas barrier
670 characteristics, but poor water barrier characteristics due to their hydrophilic character. Conversely,
671 lipids, as hydrophobic compounds, provide an appropriate barrier to moisture and also gloss to
672 enhance the appearance of coated produce. These are the basic components, but plasticizers and
673 emulsifiers or surfactants are often also added as matrix components to improve different
674 characteristics of the emulsion. These matrixes may be directly used as coatings or be carriers of
675 additional ingredients added to widen the emulsion functionality (Han, 2014). In the particular case
676 of antifungal edible coatings, the additional ingredients are food-grade compounds of different nature
677 with proven antifungal properties (Valencia-Chamorro et al., 2011).

678 Incorporating essential oils or plant extracts into composite edible coatings can be a viable
679 option for addressing some of the problems that have been witnessed when applying essential oils for
680 delaying disease symptoms of fresh fruit and vegetables. This allows the control of the pathogens,
681 while regulating the diffusion process of the volatile constituents of the essential oil (Shao et al.,
682 2015). Moreover, essential oils have been incorporated into edible coatings for enhancing the coating

683 properties. They have the potential to extend the shelf life of the produce, maintaining the desirable
684 quality attributes of the produce (Sung et al., 2013). The combination of numerous elements can
685 prove beneficial for the quality and shelf life of the plant product. Edible coatings have been
686 developed, which explore the combination of essential oils with polysaccharide and lipid based
687 composite coatings. On citrus fruits, although research has focused mainly on the incorporation of
688 essential oils into commercial waxes (not edible), few works investigated the addition of natural
689 antifungal plant compounds to edible hydrocolloid-lipid formulations. Thus, hydroxypropylmethyl
690 cellulose (HPMC)-lipid films formulated with different concentrations of an ethanolic extract of
691 propolis effectively inhibited the pathogen *P. italicum* in in vitro tests (Pastor et al., 2010).
692 Carboxymethyl cellulose (CMC) coatings containing essential oil from *Impatiens balsamina*
693 significantly reduced natural decay on long-term stored oranges (Zeng et al., 2013). Similarly,
694 *Penicillia* molds were significantly controlled on artificially inoculated oranges treated with a pectin-
695 based edible coating amended with essential oil at different concentrations (Velásquez et al., 2014).
696 The use of pullulan coating enriched with *Satureja hortensis* extracts on pepper and apple decreased
697 weight loss and improved fruit quality parameters during 2-4 weeks of storage, together with an
698 antimicrobial activity against some foodborne bacteria (Krasniewska et al., 2014). Working with
699 tropical fruits, Bósquez-Molina et al. (2010) investigated the role of thyme essential oil in
700 combination with mesquite gum-based and with candelilla wax on papaya, and reported 40%
701 reduction in decay caused by *R. stolonifer* and 100% reduction in decay caused by *C.*
702 *gloeosporioides*. The combination of 0.4% cinnamon essential oil with 10% gum Arabic was
703 explored by Maqbool et al. (2011) for controlling anthracnose of banana and papaya. The authors
704 reported 80% and 71% lower incidence of decay caused by *C. musae* and *C. gloeosporioides*,
705 respectively. The activity was ascribed to the presence of the essential oils, since gum Arabic alone
706 did not exhibit antimicrobial properties.

707 Another group of antifungal compounds that have been explored as ingredients of edible
708 composite coatings is comprised of synthetic food additives or GRAS substances such as some
709 inorganic and organic acids and their salts. In extensive work with citrus fruits conducted in Valencia
710 (Spain), a variety of HPMC-beeswax edible composite films containing PS, SB, SP, SMP, and some
711 mixtures of these preservatives as the most effective salts, was developed and tested on
712 commercially important orange and mandarin cultivars for the control of green and blue molds
713 (Valencia-Chamorro et al., 2008, 2009, 2011). The efficacy and overall performance of the coatings
714 was strongly dependent on the susceptibility of each citrus cultivar to decay caused by *Penicillium*
715 spp., and it was generally higher on oranges than on mandarins. This result could be ascribed to a
716 thinner and less reactive skin of mandarins as compared to oranges. In general, the coatings reduced

717 fruit weight loss and maintained firmness without adverse effects on the overall sensory quality of
718 coated fruit. Although not covered in this review, commercial citrus waxes amended with GRAS
719 salts instead of conventional fungicides have also been evaluated against citrus postharvest diseases.
720 Among a number of common preservative salts, PS, AC, ABC, and paraben salts incorporated into
721 HPMC-lipid coatings showed the best performance to control brown rot caused by *M. fructicola* and
722 preserve postharvest quality of plums (Karaca et al., 2014). Polysaccharide edible coatings based on
723 guar gum or pea starch formulated with PS significantly reduced decay caused by several postharvest
724 pathogens on apples, cucumbers, and tomatoes (Mehyar et al., 2011). Fagundes et al. (2013) reported
725 that HPMC-lipid materials formulated with antifungal GRAS salts controlled black spot of cherry
726 tomato caused by *A. alternata* more effectively than gray mold caused by *B. cinerea*. The best results
727 for reduction of gray mold were obtained with materials containing 2% PC, PBC, AC, or ammonium
728 phosphate (AP), while 2% sodium paraben salts were the best ingredients for coatings against black
729 rot. Food preservatives selected from previous research included PC, AP, AC, and sodium
730 propionate (SP) (Fagundes et al., 2014). All antifungal compounds significantly reduced gray mold
731 development on inoculated and cold-stored cherry tomatoes, the SP-based compound being the most
732 effective. The AC-based compound was the most effective to control weight loss and maintain the
733 firmness of coated fruit. Respiration rate, firmness, color, sensory flavor, off-flavor, and fruit
734 appearance were not adversely affected by the application of the antifungal coatings.

735

736 **6. Concluding remarks**

737 Due to the high economic value of worldwide trade of fresh horticultural produce and the
738 important problems related to conventional fungicides, the development of novel and
739 environmentally-friendly physical, biological, and chemical methods for postharvest disease control
740 is a very active research field on many public and private institutions all over the world. As
741 highlighted in the present review, a considerable number of investigations describing new low-
742 toxicity chemical methods and reporting interesting results for decay control is available from the
743 specialized literature and will certainly increase in the next few years. GRAS salts, plant extracts,
744 peptides, and natural and synthetic edible coatings with proven antifungal properties have been
745 evaluated against many important postharvest pathogens of temperate, subtropical and tropical fruit,
746 and fruit-like vegetables. With this intensive research work, the possibility of identifying new potent
747 antifungal compounds and developing suitable non-polluting chemical alternatives for commercial
748 marketing appears to be bright.

749 Despite this substantial progress, the general commercial implementation of alternative
750 chemicals is still limited, first because of the current availability of highly effective, convenient, and

751 cheaper conventional fungicides, and second because of general limitations associated to the low
752 toxicity and edible nature of these alternatives that make unrealistic to assume that they have the
753 same fungicidal activity as conventional fungicides. Key limitations identified with the use of
754 various forms of GRAS or natural compounds include limited curative or preventive activity and
755 persistence and too narrow spectrum of action. Inconsistent results are often reported, that differ
756 depending on the type of crop or nature of disease or storage conditions. This has wide implications
757 in terms of commercializing such technologies, as it is apparent that most applications need to be
758 tailor-made for specific plant products. It should be taken into account, in this sense, that fruit export
759 markets require minimal disease incidence and, in contrast to conventional chemical fungicides that
760 directly kill the target pathogen, alternative means have often a rather fungistatic mode of action.
761 Thus, their effectiveness is also highly dependent on fruit host characteristics such as species and
762 cultivar and fruit physical and physiological condition at the time of treatment, particularly peel
763 condition and ripening stage. Moreover, some natural compounds like essential oils or other plant
764 extracts with potent activity, often may have a negative impact on the flavor and aroma of treated
765 fruit or may even result in phytotoxicity, being the range among effectiveness on decay-causing
766 fungi and lack of phytotoxic effects often very limited or absent. Additionally, while in vitro studies
767 tend to be positive, it is not always the case with in vivo application of the natural compounds, as the
768 treatment may affect the fruit physiology and often may need a suitable carrier for efficient
769 application on the fruit surface.

770 Although more complex and possibly more expensive, the best strategy for overcome these
771 limitations is, besides the continuous search for new effective single compounds, the integration with
772 other low-risk treatments to optimize disease control efficacy and general performance taking
773 advantage of additive or synergistic effects. The formulation of chitosan coatings or synthetic
774 composite edible coatings with the addition of essential oils or other GRAS compounds may
775 represent an effective way to, besides improving the global functionality of the coating, reduce the
776 risks of induction of adverse sensory properties or phytotoxicity associated with the use of these
777 ingredients as stand-alone gaseous or aqueous treatments. Furthermore, coating application can also
778 increase the antifungal activity of the ingredient by regulating its temporal and spatial release or
779 facilitating its continuous and effective contact with the target pathogen. The integrated approach,
780 however, should not be limited to combinations of postharvest treatments. Cost-effective postharvest
781 disease control in the absence of conventional fungicides requires the implementation of global IDM
782 programs that take into account all preharvest, harvest, and postharvest factors that may influence
783 disease incidence. The purpose of these programs, based on comprehensive knowledge of the aspects
784 defining the disease triangle, i.e. pathogen, fruit host, and environment, is to define all the actions

785 needed during the entire fruit production cycle to minimize final economic losses due to decay. In
786 the development of these multifaceted strategies, emphasis should be placed on minimizing human
787 health risks and environmental toxicity.

788

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792

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