This is the peer reviewd version of the followng article:

Edible coatings incorporating pomegranate peel extract and biocontrol yeast to reduce Penicillium digitatum postharvest decay of oranges / Kharchoufi, S.; Parafati, L.; Licciardello, F.; Muratore, G.; Hamdi, M.; Cirvilleri, G.; Restuccia, C.. - In: FOOD MICROBIOLOGY. - ISSN 0740-0020. - 74:(2018), pp. 107-112. [10.1016/j.fm.2018.03.011]

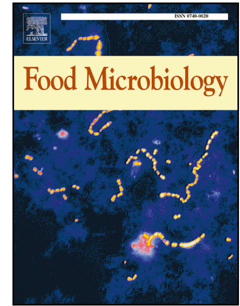
Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

12/09/2024 07:40

(Article begins on next page)

Accepted Manuscript



Edible coatings incorporating pomegranate peel extract and biocontrol yeast to reduce *Penicillium digitatum* postharvest decay of oranges

Samira Kharchoufi, Lucia Parafati, Fabio Licciardello, Giuseppe Muratore, Mokthar Hamdi, Gabriella Cirvilleri, Cristina Restuccia

PII: S0740-0020(17)31064-X

DOI: 10.1016/j.fm.2018.03.011

Reference: YFMIC 2982

To appear in: *Food Microbiology*

Please cite this article as: Samira Kharchoufi, Lucia Parafati, Fabio Licciardello, Giuseppe Muratore, Mokthar Hamdi, Gabriella Cirvilleri, Edible coatings incorporating pomegranate peel extract and biocontrol yeast to reduce *Penicillium digitatum* postharvest decay of oranges, *Food Microbiology* (2018), doi: 10.1016/j.fm.2018.03.011

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

1 **Edible coatings incorporating pomegranate peel extract and biocontrol yeast to reduce**
2 ***Penicillium digitatum* postharvest decay of oranges**
3

4 Samira Kharchoufi[a], Lucia Parafati[b], Fabio Licciardello[c], Giuseppe Muratore[b], Mokthar
5 Hamdi[a], Gabriella Cirvilleri[b], Cristina Restuccia[b,] *
6
7 [a] *Laboratory of Microbial Ecology and Technology, National Institute of Applied Sciences*
8 *and Technology (INSAT), University of Carthage, Centre Urbain Nord, 2 Boulevard de la*
9 *Terre, B.P. 676, 1080 Tunis, Tunisia*
10 [b] *Department of Agriculture, Food and Environment (Di3A), University of Catania, via S.*
11 *Sofia 100, 95123, Catania, Italy*
12 [c] *Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2,*
13 *42122 Reggio Emilia, Italy*
14
15
16
17
18
19
20
21
22
23
24
25 * Corresponding author. Tel.:+39 095 7580219
26 *E-mail address*: crestu@unict.it (C. Restuccia).
27

ACCEPTED MANUSCRIPT

28

29

30 ABSTRACT

31 This study investigated the potential use of two edible coatings, chitosan (CH) and locust
32 bean gum (LBG), which incorporated chemically characterized water pomegranate peel
33 extract (WPPE) or methanol pomegranate peel extract (MPPE) and the biocontrol agent
34 (BCA) *Wickerhamomyces anomalus,* to control the growth of *Penicillium digitatum* and to
35 reduce the postharvest decay of oranges.

36 CH and LBG including pomegranate peel extracts (PPEs) at different concentrations were
37 tested *in vitro* against *P. digitatum* to determine their antifungal efficacy; at the same time, the
38 tolerance of viable cells of *W. anomalus* to increasing concentrations of WPPE and MPPE
39 extracts was assessed. The potential application of selected bioactive coatings was evaluated
40 *in vivo* on oranges, which had been artificially inoculated with *P. digitatum*, causal agent of
41 green mold decay. CH incorporating MPPE or WPPE at all concentrations was able to inhibit
42 *in vitro P. digitatum*, while LBG was active only at the highest MPPE or WPPE
43 concentrations. *W. anomalus* BS91 was slightly inhibited only by MPPE-modified coatings,
44 while no inhibition was observed by WPPE, which was therefore selected for the *in vivo* trials
45 on oranges artificially inoculated with *P. digitatum*. The experimental results proved that the
46 addition of 0.361 g dry WPPE/mL, both to CH and LBG coatings, significantly reduced
47 disease incidence (DI) by 49 and 28% respectively, with respect to the relative controls.
48 Besides the combination CH or LBG + WPPE, the addition of *W. anomalus* cells to coatings
49 strengthened the antifungal effect with respect to the relative controls, as demonstrated by the
50 significant reduction of DI (up to 95 and 75% respectively). The findings of the study
51 contribute to the valorization of a value-added industrial byproduct and provide a significant

52 advancement in the development of new food protectant formulations, which benefit from the
53 synergistic effect between biocontrol agents and natural bioactive compounds.
54

55 *Keywords*: Locust bean gum; Chitosan; *Wickerhamomyces anomalus*; Disease parameters
56

57 1. Introduction
58

59 Fruit and vegetables are an important part of a healthy diet, due to their high content of
60 vitamins, minerals and antioxidant compounds. Unfortunately, they are highly perishable and,
61 during the postharvest stages, up to 25 and 50% of the total production in, respectively,
62 industrialized and developing countries can be lost due to fungal pathogens (Li Destri Nicosia
63 et al., 2016). Furthermore, fungal proliferation may lead to the contamination of products with
64 mycotoxins as secondary metabolites (Wu et al., 2014).

65 The investigation of new eco-friendly approaches, such as the application of antagonistic
66 microorganisms (Liu et al., 2013; Panebianco et al., 2015; Restuccia et al., 2006; Ruiz-
67 Moyano et al., 2016; Scuderi et al., 2009) and/or of natural antimicrobial substances (Aloui et
68 al., 2014; Palou et al., 2016) to control the postharvest mold decay, is preferred to synthetic
69 fungicides to prevent negative or adverse effects on human health and nature balance. Such
70 alternative solutions are considered relatively safe due to their natural origin, biodegradability
71 and low toxicity to the environment (da Cruz Cabral et al., 2013). In particular, a biological
72 control which relies on antagonistic yeasts has been reported to be effective at managing
73 postharvest decay of a variety of fruit (Liu et al., 2013). Many yeasts belonging the genera
74 *Candida*, *Metschnikowia*, *Wickerhamomyces* and *Aureobasidium* have been reported as
75 effective biocontrol agents (BCAs) on postharvest diseases of citrus, apple, pear, grapefruit,
76 table grape and sweet cherry (Aloui et al., 2015; Lutz et al., 2013; Oro et al., 2014; Parafati et

ACCEPTED MANUSCRIPT

77 al., 2015; Platania et al., 2012). In the domain of plant-derived compounds with antimicrobial
78 potential, PPE has been extensively investigated for its free radical scavenging effect and
79 strong antioxidant capacity caused by the high concentration of biologically active
80 components, such as punicalagin, ellagic, gallic and chlorogenic acids (Elsherbiny et al.,
81 2016; Kazemi et al., 2016; Kharchoufi et al., 2018). Few researches investigated extracts from
82 pomegranate peel as natural inhibitors for plant pathogenic bacteria and fungi (Elsherbiny et
83 al., 2016; Endo et al., 2010; Romeo et al., 2015), including *Penicillium digitatum* (Kharchoufi
84 et al., 2018; Li Destri Nicosia et al., 2016). However, there are still major obstacles to the
85 large-scale use of plant extracts to control postharvest pathogens. Although plant extracts
86 have proved to be good antimicrobial agents, their use for maintaining fruit quality and
87 reducing fungal decay is often limited by application costs, reduced and inconsistent efficacy
88 as a result of fruit physiology and environment, low residual activity, lack of curative effect
89 and limited range of activity against different fungal pathogens (Bautista-Banos, 2014).

90 The incorporation of these natural compounds into edible coating formulations can be an
91 effective approach to solve some of these problems while, at the same time, controlling fruit
92 postharvest decay by lowering the diffusion processes and maintaining high concentrations of
93 active molecules at the fruit surface. Edible coatings could be considered a safer alternative
94 solution for citrus fruit than wax coatings (Parafati et al., 2016), which usually are composed
95 of oxidized polyethylene, organic solvents, surfactants and preservatives such as sodium
96 methylparaben (Moscoso-Ramírez et al., 2013). Edible films and coatings, mainly constituted
97 by starch, cellulose derivatives, chitosan/chitin, gums, proteins (animal or vegetable) and
98 lipids, have been developed as natural or nonpolluting materials to replace commonly used
99 waxes to extend the shelf-life of fruit, to improve fruit appearance, to reduce moisture losses,
100 and eventually to incorporate antimicrobial food additives (Aloui et al., 2014; Valencia-
101 Chamorro et al., 2011; Zhang et al., 2016a,b). Chitosan coatings have been widely reported to

ACCEPTED MANUSCRIPT

102 limit fungal decay and to delay the ripening of several commodities, including dates (Aloui et
103 al., 2014), table grape (Guerra et al., 2016), and citrus fruit (Panebianco et al., 2014).
104 Moreover, chitosan-based coatings can be used as a carrier to incorporate functional
105 ingredients, such as antimicrobials, antioxidant enzymes, minerals, vitamins, and
106 antioxidants. Locust bean gum (LBG) is a polysaccharide widely used in the production of
107 edible films/coatings due to its edibility, biodegradability and hydrophilic properties (Barak
108 and Mudgil, 2014; Sébastien et al., 2014), dissolved in water solutions. LBG in edible films
109 and coatings may also act as carrier of additives, bioactive compounds (Aloui et al., 2014) and
110 biocontrol agents (Aloui et al., 2015; Parafati et al., 2016) whose viability over time can be
111 accordingly extended.

112 To the best of our knowledge, no research has been performed on edible coatings enriched
113 with PPE in combination with biocontrol yeasts and their application in the postharvest
114 preservation of oranges. Therefore, the purpose of the study is to: *i*) screen *in vitro* the
115 antifungal potential of two edible coatings, CH and LBG, enriched with different
116 combinations of chemically characterized water and methanol PPEs, and *ii*) evaluate the most
117 effective formulations, enriched or not with BCA *W. anomalus*, on artificially inoculated
118 oranges, to validate *in vivo* the combined biocontrol strategy.

119

120 **2. Materials and methods**

121 *2.1. Microorganisms and culture conditions*

122 *Penicillium digitatum* and *Wickerhamomyces anomalus* BS91 strains belong to the Di3A
123 (Dipartimento di Agricoltura, Alimentazione e Ambiente, University of Catania, Italy)
124 collection. *W. anomalus* was previously selected for its good antagonistic ability related to β-
125 glucanase production (Muccilli et al., 2013; Parafati et al., 2016). More recently, bioactive

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

126 coatings enriched with *W. anomalus* BS91, exhibited a good control of *P. digitatum* on
127 mandarin and orange (Aloui et al., 2015; Parafati et al., 2016).
128 The mold and yeast stock cultures were stored at 4 °C on Petri dishes containing, respectively,
129 Potato Dextrose Agar (PDA, CM0139, Oxoid, Basingstoke, UK) and Yeast Extract Peptone
130 Dextrose Agar [YPDA: yeast extract, 10 g; peptone, 10 g; dextrose, 20 g; agar, 20 g (Oxoid,
131 Basingstoke, UK) per liter of sterile distilled water (SDW)].
132

133 *2.2. Preparation of CH and LBG films carrying pomegranate peel extract (PPE)*

134 The chitosan (CH) film forming solution was prepared by dissolving CH (1%, w/v) in an
135 aqueous glacial acetic acid solution (1%, v/v), at 40 °C for 12 h. The Locust bean gum (LBG)
136 film forming solution was prepared by dissolving the LBG powder (molecular weight ~
137 310,000 Da, Sigma Aldrich, Steinheim, Germany) in distilled water (0.5%, w/v) at 70 °C
138 under constant agitation. Twenty % (w/w, based on biopolymer content) of glycerol (≥ 99%
139 purity; Sigma-Aldrich, Steinheim, Germany) was used as a plasticizer to enhance the film
140 flexibility and facilitate its release from the Petri plate.
141 The CH and LBG film forming solutions were modified by adding different amounts of
142 either a water pomegranate peel extract (WPPE) or a methanol pomegranate peel extract
143 (MPPE) (see Table 1), which had been prepared according to the procedure reported by
144 Karchoufi et al. (2018).
145

146 *2.3. In vitro antifungal activity of active films carrying PPE*

147 Ten mL of each active film forming solution (Table 1) was poured into Petri plates, and
148 dried at room temperature for approximately 48 h to produce films with a controlled

149 thickness. The films were then sterilely peeled off the Petri plates and preconditioned in
150 climatic chamber at 25 °C and 75% RH, prior to testing.

151 Disks of each film (4 mm diameter) were cut and placed on PDA plates, which had been
152 previously spray-inoculated with a conidial suspension of *P. digitatum* (adjusted at a final
153 concentration of 10^6 conidia/mL by an hemocytometer), and incubated at 25 °C for 6 days.

154 The antifungal activity was expressed as the average diameter size (mm) of fungal growth
155 inhibition zones around the bioactive film disks. The experiment was performed in triplicate
156 and repeated twice.

157

158 *2.4. W. anomalus tolerance to active films carrying PPE*

159 The potential application of BCA *W. anomalus* to active films containing PPEs has been
160 evaluated through a preliminary test. A disk of each dried active film (4 mm diameter),
161 including either WPPE or MPPE, as described in Table 1, was cut and placed on a YPDA
162 medium which had been previously inoculated according to the pour plate method with a 48-
163 h cell suspension of *W. anomalus* (final concentration 10^6 cells/mL), to test the influence of
164 the active films on the growth of *W*. anomalus. The plates were incubated at 25 °C for 72 h.

165 The growth inhibitory activity was expressed as the average diameter size (mm) of *W.*
166 *anomalus* growth inhibition zones around the bioactive film disks. The experiment was
167 performed in triplicate and repeated twice.

168

169 *2.5. Evaluation of active coatings for the control of green mold decay of wounded oranges*

170 The active coating formulations showing the best performance *in vitro* against green mold
171 and affecting less the growth of *W. anomalus* were used alone and in combination with *W.*
172 *anomalus* cells (Table 2) to conduct *in vivo* biocontrol tests on oranges which had been
173 artificially inoculated with *P. digitatum*.

ACCEPTED MANUSCRIPT

174 The yeast suspension in SDW was prepared by collecting the *W. anomalus* BS91 cells
175 grown in YPD [yeast extract, 10 g; peptone, 10 g; dextrose, 20 g (Oxoid, Basingstoke, UK)
176 per liter of SDW] for 48 h at 25 °C. The yeast suspension was incorporated into CH and LBG
177 film forming solutions at a temperature of 30 °C to achieve a final concentration of 10^8
178 cells/mL.

179 Oranges were purchased from a local organic supermarket and used within 24 h from
180 purchase. Fruits of similar size and without injuries or rot were selected for the experiments. .
181 Prior to coating, the selected oranges were washed with tap water, their surface disinfected by
182 immersion in a 11.5 g/L NaOCl solution for 3 min, rinsed with SDW and air-dried. Oranges
183 were then artificially wounded (4 wounds per fruit) with a sterile needle (3 mm diameter × 3
184 mm deep). Twenty µl of a *P. digitatum* spore suspension (10^5 conidia/mL) was inoculated into
185 each wound and dried at room temperature for 3 h to produce artificial infections. Fruits were
186 then dipped in different coating solutions for 2 min and air-dried at room temperature.
187 Uncoated oranges inoculated with *P. digitatum* were used as a control. Film coated oranges
188 were placed in a sealed plastic box to maintain a high relative humidity (90% RH) and
189 incubated at 26 °C. After 5 days of incubation, data concerning disease incidence (DI),
190 disease severity (DS) and lesion diameter (LD) were measured. In detail, DS was evaluated
191 by using an empirical 1-to-4 rating scale: 1 = no visible symptoms (0%); 2 = soft rot (35%); 3
192 = mycelium (65%); 4 = sporulation (90%) before analysis of variance. Average fruit diseade
193 severity index was calculated as reported by Parafati et al. (2015). Lesion diameter (LD) was
194 also assessed by measuring the average diameter of the damaged area five days after pathogen
195 inoculation.

196

197 *2.6. Statistical Analysis*

ACCEPTED MANUSCRIPT

198 All statistical analyses were performed using the Statistical package software Minitab™
199 version 16.0.

200 One-way analysis of variance (ANOVA) was carried out to determine statistical significant
201 ($p < 0.05$) differences among inhibition size mean values of *P. digitatum* growth for the *in*
202 *vitro* assay by the Duncan's Multiple Range test.

203 In all repeated *in vivo* experiments, DI, DS and LD were calculated, averaging the values
204 determined for the single replicates of each treatment. Within the same disease parameter (DI,
205 DS, and LD) the significant ($p < 0.05$) differences (mean separation) between treatments were
206 determined by the Duncan's Multiple Range test.

207

208 **3. Results and discussion**

209 *3.1 In vitro* evaluation of the *antifungal effectiveness of active coatings*

210 In the present study the use of bioactive CH and LBG coatings, enriched with MPPE and
211 WPPE was evaluated by means of *in vitro* assays against *P. digitatum.* Among plant-derived
212 compounds, PPE, sourced from a largely available industrial byproduct, has gained attention
213 for its antioxidant and antimicrobial properties (Tehranifar et al., 2011). The extracts used in
214 the study were characterized to determine the total phenol content and profile (Kharchoufi et
215 al., 2018), and the results demonstrated the presence of punicalagin as major component,
216 which is an ellagitannin known for its antifungal activity (Glazer et al., 2012; Romeo et al.,
217 2015).

218 Data from the antifungal assays, performed on PDA plates, showed that bioactive CH and
219 LBG coatings, enriched with PPEs, significantly inhibited the growth of *P. digitatum* by
220 producing an inhibition halo around the experimental bioactive film disks, which got larger at
221 increasing WPPE or MPPE concentrations. Overall, the highest effectiveness was obtained
222 using CH-ME3 and CH-WE3, which produced the widest inhibition zones (Table 3). Among

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

223 the LBG coatings, only LBG-ME3 or –WE3 and LBG-ME2 or –WE2 produced significant
224 inhibition halos on PDA inoculated with a *P. digitatum* conidial suspension, while no
225 inhibition was observed in the control as well as in the formulation with the lowest PPE
226 concentration (Table 3). These results are consistent with previous reports on the *in vitro*
227 antifungal effect of PPE (Glazer et al., 2012; Kharchoufi et al., 2018; Osorio et al., 2010). It
228 should be noted that neither LBG nor CH alone produced inhibition halos around the film
229 sample; however, the growth of the mycelium was not observed in the contact area between
230 CH and the agar medium, proving some intrinsic inhibitory activity of chitosan.
231 This effect was not observed for LBG alone, which, in fact, allowed the mycelium growth in
232 the area of contact with the inoculated medium.

233

234 *3.2. Influence of bioactive coatings on W. anomalus growth*

235 With the aim to develop an integrated biological control approach to effectively manage
236 postharvest *P. digitatum* decay *in vivo*, the opportunity to add a proved BCA, *W. anomalus*
237 BS91, to the bioactive WPPE- and MPPE- coatings was evaluated by a preliminary *in vitro*
238 assay. Such preliminary screening excluded any significant inhibition activity of the bioactive
239 coatings, which caused weak growth inhibition (halo of 2 mm), only at the highest MPPE
240 (0.304 g dry extract/mL) concentration. WPPE at all concentrations and MPPE at lower
241 concentrations did not produce any appreciable inhibition effect against *W. anomalus* cells,
242 nor did CH and LBG alone (no inhibition halo). Only few data regarding the effect of PPE
243 against yeasts are available. The combination of punicalagin, identified as the main active
244 antifungal compound of an hydro alcoholic extract prepared from the peel of *Punica*
245 *granatum*, with fluconazole produced a potently synergistic action, by inducing ultrastructural
246 changes against *Candida albicans* cells *in vitro* (Endo et al., 2010). The antifungal effects of
247 pomegranate pericarp and peel extracts, attributed to changes in cell morphology and

ACCEPTED MANUSCRIPT

248 structure, were demonstrated against yeasts of the *Candida* genus (Anibal et al., 2013).
249 Moreover, the crude extract of pomegranate peel showed activity against the dermatophytes
250 *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, and *Microsporum*
251 *gypseum*, with Minimum Inhibitory Concentrations (MICs) values of 125 μg/mL and 250
252 μg/mL, respectively for each genus (Foss et al., 2014). With regard to the effect of PPEs on
253 food-related yeasts, the results obtained in this study are in agreement with those recently
254 reported on *S. cerevisiae* (Kharchoufi et al., 2018). A possible explanation for this behavior
255 could be attributed to the isolation source of *W. anomalus* BS91, the olive brine, which is
256 particularly rich in plant polyphenols: this condition might have determined some adaptation
257 of the BCA *W. anomalus* to such compounds. Moreover, additional experimental evidence
258 proved that plant-derived bioactive compounds can extend the lifespan and improve
259 resistance to oxidative stress in *Saccharomyces cerevisiae* (Martorell et al., 2011).
260

261 *3.3. Evaluation of CH and LBG coatings incorporating WPPE for the control of green*
262 *mold decay on oranges*

263 The *in vitro* assays demonstrated the effectiveness of CH and LBG coatings containing
264 WPPE and MPPE against *P. digitatum* and excluded any interference on the growth of *W.*
265 *anomalus*. On the basis of such results and in consideration of the fact that food-grade and
266 environmentally-sustainable extraction methods from plant materials should be preferred,
267 especially for their use as food supplement/additive (Kharchoufi et al., 2018), water
268 pomegranate peel extract (WPPE) was selected for the subsequent *in vivo* trials on artificially
269 pathogen-inoculated oranges.
270 The results of the biocontrol activity of CH and LBG- active coatings on oranges, which had
271 been artificially inoculated with *P. digitatum*, are reported in Fig.1 and Fig. 2, respectively.
272 Both CH and LBG coatings incorporating 0.361 g dry WPPE/mL and *W. anomalus* cells

273 significantly reduced green mold decay parameters (DI, DS and LD) on oranges, providing
274 always significantly lower values if compared to the relative controls (Fig. 1 and Fig. 2).
275 Based on the widely reported role of phenolic compounds as plant defense response inducer
276 (Oliveira et al., 2016), it is likely that the postharvest application of PPE on fruit could induce
277 mechanisms of resistance against fungal pathogens (Li Destri Nicosia et al., 2016).
278 Among chitosan coatings (Fig. 1), CH-WE3-Wa displayed the highest *in vivo* effectiveness at
279 reducing DI, DS and LD values on wounded oranges (95, 98 and 100% of reduction,
280 respectively, compared to the relative control), followed by the CH-WE3 treatment (49, 65
281 and 92% of reduction, respectively, compared to the relative control). The coating treatment
282 with CH only determined an inferior reduction in the green mold decay parameters (10, 16
283 and 11% of reduction, respectively, compared to the relative control), as previously reported
284 in literature (Panebianco et al., 2014; Panebianco et al., 2016; Tayel et al., 2016). This effect
285 could be due to the morphological and structural modifications induced by chitosan on fungal
286 hyphae (Singh et al., 2008) and/or to the elicitation of biochemical defense responses in
287 coated fruit (El Guilli et al., 2016).
288 Similarly, among the essayed LBG coatings (Fig. 2) the highest effectiveness in reducing DI,
289 DS and LD values on wounded oranges was observed for the LBG-WE3-Wa combination
290 (75, 92 and 98% of reduction, respectively, compared to the relative control), followed by the
291 LBG-WE3 treatment (28, 69 and 85% of reduction, respectively, compared to the relative
292 control). Orange coating comprising only LBG did not determine any appreciable reduction in
293 the green mold decay in comparison with the relative control.
294 Overall, the highest reductions of the green mold diseases parameters were obtained by using
295 CH-WE3-Wa followed by LBG-WE3-Wa (Fig. 3), which almost completely inhibited rot
296 development on oranges.
297 The demonstrated efficacy of PPEs *in vivo* is consistent with the results reported by Li Destri

ACCEPTED MANUSCRIPT

298 Nicosia et al. (2016) who observed that the treatment with PPE at 12 g/L on lemons, 6 h after
299 pathogen inoculation, resulted in the reduction of *P. digitatum* infection by 76%, while a level
300 of 1.2 g/L determined a reduction of 46.7%. On grapefruit, PPE at 12 or 1.2 g/L reduced *P.*
301 *digitatum* infection by 68.9% and 44.8%, respectively. Furthermore, the addition of BCA *W.*
302 *anomalus* BS91 yeast, with a proved efficacy against *P. digitatum* on citrus fruit (Platania et
303 al., 2012; Aloui et al., 2015; Parafati et al., 2016), to the CH and LBG bioactive coatings,
304 always boosted the activity against *P. digitatum* on orange fruit, allowing the largest
305 reductions of decay parameters.

306

307 **4. Conclusions**

308 Since harvested fruit and vegetables are of high value, the development of integrated and
309 increasing efficient control strategies which can reduce the loss of products is still a research
310 priority.

311 The present study provides evidence of the high potential of bioactive CH and LBG
312 coatings enriched with WPPE as a natural, safe and eco-friendly postharvest control strategy.
313 The WPPE incorporated in edible coating matrices determined a good level of inhibition of
314 green mold on artificially inoculated oranges. Such results are very promising, especially in
315 consideration of the fact that the conditions evaluated in the study are by far worse than those
316 normally occurring during the postharvest life, both in terms of fruit injury degree and
317 pathogen inoculum level. In addition, the results proved, for the first time, the antifungal
318 effectiveness of WPPE in combination with biocontrol yeast *W. anomalus*. The tolerance of
319 yeasts to pomegranate bioactive compounds is a feature which could be exploited in various
320 fields, such as the production of fermented foods, where bioactive compounds extracted from
321 pomegranate peel could replace potentially harmful synthetic preservatives. Moreover, the

ACCEPTED MANUSCRIPT

322 specific tolerance of biocontrol yeast *W. anomalus* BS91 to PPE, opens interesting strategies
323 in the integrated management of postharvest decay and foreseeing advances in the
324 development of new formulations which leverage on the synergy between biocontrol agents
325 and natural bioactive compounds.

326

327 **Aknowledgements**

328

329 International mobility of Samira Kharcoufi was supported by the European Union funding
330 (PASRI).

331

ACCEPTED MANUSCRIPT

332 **References**

333 Aloui, H., Khwaldia, K., Licciardello, F., Mazzaglia, A., Muratore, G., Hamdi, M., Restuccia,
334 C., 2014. Efficacy of the combined application of chitosan and Locust Bean Gum with
335 different citrus essential oils to control postharvest spoilage caused by *Aspergillus flavus*
336 in dates. Int. J. Food Microbiol. 170, 21–28.

337 Aloui, H., Licciardello, F., Khwaldia, K., Hamdi, M., Restuccia, C., 2015. Physical properties
338 and antifungal activity of bioactive films containing *Wickerhamomyces anomalus* killer
339 yeast and their application for preservation of oranges and control of postharvest green
340 mold caused by *Penicillium digitatum*. Int. J. Food Microbiol. 200, 22–30.

341 Anibal, P.C., Peixoto, I.T.A., Foglio, M.A., Höfling, J.F., 2013. Antifungal activity of the
342 ethanolic extracts of *Punica granatum* L. and evaluation of the morphological and
343 structural modifications of its compounds upon the cells of *Candida* spp. Braz. J.
344 Microbiol. 44, 839–848.

345 Barak, S., Mudgil, D., 2014. Locust bean gum: processing, properties and food applications--a
346 review. Int. J. Biol. Macromol. 66, 74–80.

347 Bautista-Banos, S., Sivakumar, D., Bello-Pérez, A., Villanueva-Arce, R., Hernández-López,
348 M., 2013. A review of the management alternatives for controlling fungi on papaya fruit
349 during the postharvest supply chain. Crop Prot. 49, 8–20.

350 da Cruz Cabral, L., Fernández Pinto, V., Patriarca, A., 2013. Application of plant derived
351 compounds to control fungal spoilage and mycotoxin production in foods. Int. J. Food
352 Microbiol. 166, 1–14.

353 El Guilli, M., Hamza, A., Clément, C., Ibriz, M., Ait Barka, E., 2016. Effectiveness of
354 postharvest treatment with chitosan to control citrus green mold. Agriculture 6, 12.

355 Elsherbiny, E.A., Amin, B.H., Baka, Z.A., 2016. Efficiency of pomegranate (*Punica*
356 *granatum* L.) peels extract as a high potential natural tool towards *Fusarium* dry rot on

ACCEPTED MANUSCRIPT

357 potato tubers. Postharvest Biol. Technol. 111, 256–263.

358 Endo, E.H., Garcia Cortez, D.A., Ueda-Nakamura, T., Nakamura, C.V., Dias Filho, B.P.,
359 2010. Potent antifungal activity of extracts and pure compound isolated from
360 pomegranate peels and synergism with fluconazole against *Candida albicans*. Res.
361 Microbiol. 161, 534–540.

362 Foss, S.R., Nakamura, C.V., Ueda-Nakamura, T., Cortez, D.A.G., Endo, E.H., Dias Filho,
363 B.P., 2014. Antifungal activity of pomegranate peel extract and isolated compound
364 punicalagin against dermatophytes. Ann. Clin. Microbiol. Antimicrob. 13, 32.

365 Glazer, I., Masaphy, S., Marciano, P., Bar–Ilan, I., Holland, D., Kerem, Z., Amir, R., 2012.
366 Partial identification of antifungal compounds from *Punica granatum* peel extracts. J.
367 Agric. Food Chem. 60, 4841–4848.

368 Guerra, I.C.D., de Oliveira, P.D.L., Santos, M.M.F., Lúcio, A.S.S.C., Tavares, J.F., Barbosa-
369 Filho, J.M., Madruga, M.S., de Souza, E.L., 2016. The effects of composite coatings
370 containing chitosan and *Mentha* (*piperita* L. or x *villosa* Huds) essential oil on
371 postharvest mold occurrence and quality of table grape cv. Isabella. Innov. Food Sci.
372 Emerg. Technol. 34, 112–121.

373 Kharchoufi, S., Licciardello, F., Siracusa, L., Muratore, G., Hamdi, M., Restuccia, C., 2018.
374 Antimicrobial and antioxidant features of 'Gabsi' pomegranate peel extracts. Ind. Crops
375 Prod. 111, 345-352.

376 Kazemi, M., Karim, R., Mirhosseini, H., Abdul Hamid, A., 2016. Optimization of pulsed
377 ultrasound-assisted technique for extraction of phenolics from pomegranate peel of
378 Malas variety: Punicalagin and hydroxybenzoic acids. Food Chem. 206, 156–166.

379 Li Destri Nicosia, M.G., Pangallo, S., Raphael, G., Romeo, F. V., Strano, M.C., Rapisarda, P.,
380 Droby, S., Schena, L., 2016. Control of postharvest fungal rots on citrus fruit and sweet
381 cherries using a pomegranate peel extract. Postharvest Biol. Technol. 114, 54–61.

ACCEPTED MANUSCRIPT

382 Liu, J., Sui, Y., Wisniewski, M., Droby, S., Liu, Y., 2013. Review: Utilization of antagonistic
383 yeasts to manage postharvest fungal diseases of fruit. Int. J. Food Microbiol. 167, 153–
384 160.
385 Lutz, M.C., Lopes, C.A., Rodriguez, M.E., Sosa, M.C., Sangorrín, M.P., 2013. Efficacy and
386 putative mode of action of native and commercial antagonistic yeasts against postharvest
387 pathogens of pear. Int. J. Food Microbiol. 164, 166–172.
388 Martorell, P., Forment, J.V., de Llanos, R., Monton, F., Llopis, S., Gonzalez, N., Genoves, S.,
389 Cienfuegos, E., Monzo, H., Ramon, D., 2011. Use of *Saccharomyces cerevisiae* and
390 *Caenorhabditis elegans* as model organisms to study the effect of cocoa polyphenols in
391 the resistance to oxidative stress. J. Agric. Food Chem. 59, 2077–2085.
392 Moscoso-Ramírez, P.A., Montesinos-Herrero, C., Palou, L., 2013. Characterization of
393 postharvest treatments with sodium methylparaben to control citrus green and blue
394 molds. Postharvest Biol. Technol. 77, 128–137.
395 Muccilli, S., Sabrina, W., Cristina, R., Meinhardt, F., 2013. Exoglucanase-encoding genes
396 from three *Wickerhamomyces anomalus* killer strains isolated from olive brine. Yeast 30,
397 33–43.
398 Oliveira, M.D.M., Varanda M.R.F., Felix, M.R.F., 2016. Induced resistance during interaction
399 pathogen x plant and the use of resistance inducer. Phytochem. Lett. 15, 152–158.
400 Oro, L., Feliziani, E., Ciani, M., Romanazzi, G., Comitini, F., 2014. Biocontrol of postharvest
401 brown rot of sweet cherries by *Saccharomyces cerevisiae* Disva 599, *Metschnikowia*
402 *pulcherrima* Disva 267 and *Wickerhamomyces anomalus* Disva 2 strains. Postharvest
403 Biol. Technol. 96, 64–68.Osorio, E., Floresa, M., Hernàndeza, D., Venturab, J.,
404 Rodrìguezb, R., Aguilarb, C.N., 2010. Biological efficiency of polyphenolic extracts
405 from pecan nuts shell (*Carya Illinoensis*), pomegranate husk (*Punica granatum*) and
406 creosote bush leaves (*Larrea tridentata* Cov.) against plant pathogenic fungi. Ind. Crops

407 Prod. 31, 153– 157.

408 Palou, L., Ali, L., Fallik, E., Romanazzi, G., 2016. GRAS, plant- and animal-derived
409 compounds as alternatives to conventional fungicides for the control of postharvest
410 dieseases of fresh horticultural produce. Postharvest Biol. Technol. 122, 41–52.

411 Panebianco S., Vitale A., Platania C, Restuccia C., Polizzi G., Cirvilleri G., 2014. Postharvest
412 efficacy of resistance inducers for the control of green mold on important Sicilian
413 citrus varieties. J. Plant Dis. Prot. 121, 177–183.

414 Panebianco, S., Vitale, A., Polizzi, G., Scala, F., Cirvilleri, G., 2015. Enhanced control of
415 postharvest citrus fruit decay by means of the combined use of compatible biocontrol
416 agents. Biol. Control 84, 19–27.

417 Panebianco S., Vitale A., Polizzi G., Restuccia C., Cirvilleri G. 2016. Control of *Penicillium*
418 *digitatum* on “Tarocco” orange by combined application of *Pseudomonas syringae*
419 and resistance inducers. Acta Hortic. 1, 431–438.

420 Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2015. Biocontrol ability and action
421 mechanism of food-isolated yeast strains against *Botrytis cinerea* causing postharvest
422 bunch rot of table grape. Food Microbiol. 47, 85–92.

423 Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2016. The effect of locust bean gum
424 (LBG)-based edible coatings carrying biocontrol yeasts against *Penicillium digitatum*
425 and *Penicillium italicum* causal agents of postharvest decay of mandarin fruit. Food
426 Microbiol. 58, 87–94.

427 Platania, C., Restuccia, C., Muccilli, S., Cirvilleri, G., 2012. Efficacy of killer yeasts in the
428 biological control of *Penicillium digitatum* on Tarocco orange fruits (*Citrus sinensis*).
429 Food Microbiol. 30, 219–225.

430 Restuccia, C., Giusino, F., Licciardello, F., Randazzo, C., Caggia, C., Muratore, G., 2006.
431 Biological control of peach fungal pathogens by commercial products and indigenous

ACCEPTED MANUSCRIPT

432 yeast. J. Food Prot. 69, 2465–2470.

433 Romeo, F.V., Ballistreri, G., Fabroni, S., Pangallo, S., Li Destri Nicosia, M.G., Schena, L.,
434 Rapisarda, P., 2015. Chemical characterization of different sumac and pomegranate
435 extracts effective against *Botrytis cinerea* rots. Molecules 20, 11941–11958.

436 Ruiz-Moyano, S., Martín, A., Villalobos, M.C., Calle, A., Serradilla, M.J., Córdoba, M.G.,
437 Hernández, A., 2016. Yeasts isolated from figs (*Ficus carica* L.) as biocontrol agents of
438 postharvest fruit diseases. Food Microbiol. 57, 45–53.

439 Scuderi, G., Bonaccorsi, A., Panebianco, S., Vitale, A., Polizzi, G., Cirvilleri, G., 2009. Some
440 strains of *Burkholderia gladioli* are potential candidates for postharvest biocontrol of
441 fungal rots in citrus and apple fruits. J. Plant Pathol. 91, 207–213.

448 Sébastien, G., Christophe, B., Mario, A., Pascal, L., Michel, P., Aurore, R., 2014. Impact of
449 purification and fractionation process on the chemical structure and physical properties
450 of locust bean gum. Carbohydr. Polym. 108, 159–68.

451 Singh, T., Vesentini, D., Singh, A.P., Daniel, G., 2008. Effect of chitosan on physiological,
452 morphological, and ultrastructural characteristics of wood-degrading fungi. Int.
453 Biodeterior. Biodegrad. 62, 116–124.

454 Tehranifar, A., Selahvarzia, Y., Kharrazia, M., Bakhshb, V.J., 2011. High potential of agro-
455 industrial by-products of pomegranate (*Punica granatum* L.) as the powerful antifungal
456 and antioxidant substances. Ind. Crop. Prod. 34, 1523–1527.

457 Valencia-Chamorro, S.A., Palou, L., del Rio, M.A., Pérez-Gago, M.B., 2011. Antimicrobial
458 Edible Films and Coatings for Fresh and Minimally Processed Fruits and Vegetables: A
459 Review. Int. J. Food Microbiol. 134, 244–248.

460 Wu, F., Groopman, J.D., Pestka, J.J., 2014. Public health impacts of foodborne mycotoxins.
461 Annu. Rev. Food Sci. Technol. 5, 351–372.

462 Zhang, H., Yang, Q., Ge, L., Zhang, G., Zhang, X., Zhang, X., 2016a. Chitin enhances

ACCEPTED MANUSCRIPT

463 biocontrol of *Rhodotorula mucilaginosa* to postharvest decay of peaches. Int. J. Biol.
464 Macromol. 88, 465–475.
465 Zhang, L., Li, S., Dong, Y., Zhi, H., Zong, W., 2016b. Tea polyphenols incorporated into
466 alginate-based edible coating for quality maintenance of Chinese winter jujube under
467 ambient temperature. LWT - Food Sci. Technol. 70, 155–161.
468

ACCEPTED MANUSCRIPT

469 **Table 1**

470 Bioactive film formulations used in the *in vitro* experiments.

Coating code	Composition
CH	Chitosan
CH-WE1	chitosan + 0.072 g dry water pomegranate peel extract/mL
CH-WE2	chitosan + 0.180 g dry water pomegranate peel extract/mL
CH-WE3	chitosan + 0.361 g dry water pomegranate peel extract/mL
CH-ME1	chitosan + 0.061 g dry methanol pomegranate peel extract/mL
CH-ME2	chitosan + 0.152 g dry methanol pomegranate peel extract/mL
CH-ME3	chitosan + 0.304 g dry methanol pomegranate peel extract/mL
LBG	locust bean gum
LBG-WE1	locust bean gum + 0.072 g dry water pomegranate peel extract/mL
LBG-WE2	locust bean gum + 0.180 g dry water pomegranate peel extract/mL
LBG-WE3	locust bean gum + 0.361 g dry water pomegranate peel extract/mL
LBG-ME1	locust bean gum + 0.061 g dry methanol pomegranate peel extract/mL
LBG-ME2	locust bean gum + 0.152 g dry methanol pomegranate peel extract/mL
LBG-ME3	locust bean gum + 0.304 g dry methanol pomegranate peel extract/mL

471

472

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

473 **Table 2**

474 Bioactive coating formulations essayed in the *in vivo* test on oranges.

Coating code	Composition
CH	Chitosan
CH-WE3	chitosan + 0.361 g dry water pomegranate peel extract/mL
CH-WE3-Wa	chitosan + 0.361 g dry water pomegranate peel extract/mL + 10^8 cell/mL *W. anomalus*
LBG	locust bean gum
LBG-WE3	locust bean gum + 0.361 g dry water pomegranate peel extract/mL
LBG-WE3-Wa	locust bean gum + 0.361 g dry water pomegranate peel extract/mL + 10^8 cell/mL *W. anomalus*

475

476

ACCEPTED MANUSCRIPT

477

Table 3

Growth inhibition size (mm) of *P. digitatum* by different bioactive coatings.

Bioactive formulation	Inhibition size (mm)	Bioactive formulation	Inhibition size (mm)
CH	0.0 ± 0.00f *	LBG	0.0 ± 0.00e*
CH-WE1	0.5 ± 0.29e	LBG-WE1	0.3 ± 0.25e
CH-WE2	1.3 ± 0.14d	LBG-WE2	1.0 ± 0.00cd
CH-WE3	3.1 ± 0.13b	LBG-WE3	2.3 ± 0.14b
CH-ME1	1.3 ± 0.29d	LBG-ME1	0.5 ± 0.29de
CH-ME2	2.1 ± 0.13c	LBG-ME2	1.3 ± 0.14c
CH-ME3	4.1 ± 0.13a	LBG-ME3	3.3 ± 0.14a

478 Data are presented as mean of 3 replicate ± SE. Among bioactive formulation (CH or LBG) inhibition size
479 values followed by the same letter are not significantly different according to Duncan's test ($p < 0.05$). * It
480 should be noticed that, even though neither of the films produced a visible halo around the disk sample, CH,
481 unlike LBG, inhibited the fungal growth in the area of contact with the agar medium.

482

ACCEPTED MANUSCRIPT

483 **Figure legends**

484

485 **Fig. 1.** Biocontrol effectiveness of chitosan coating incorporating 0.361 g dry WPPE /mL,
486 alone or in combination with *W. anomalus* cells, against *Penicillium digitatum* on oranges.
487 For treatments codification, please refer to Table 2. Bars indicate standard error of the mean.
488 Columns within each disease parameter (DI: disease incidence; DS: disease severity; LD:
489 lesion diameter) followed by the same letter are not significantly different according to
490 Duncan's test ($p < 0.05$).

491

492 **Fig. 2.** Biocontrol effectiveness of LBG coating incorporating 0.361 g dry WPPE /mL, alone
493 or in combination with *W. anomalus* cells, against *Penicillium digitatum* on oranges. For
494 treatments codification, please refer to Table 2. Bars indicate standard error of the mean.
495 Columns within each disease parameter (DI: disease incidence; DS: disease severity; LD:
496 lesion diameter) followed by the same letter are not significantly different according to
497 Duncan's test ($p < 0.05$).

498

499 **Fig. 3.** Visual effect of the application of CH and LBG coatings, incorporating 0.361 g dry
500 WPPE/mL and 10^8 cells/mL *W. anomalus*, on oranges artificially inoculated with *Penicillium*
501 *digitatum* after incubation at 26 °C for 5 days.

502

ACCEPTED MANUSCRIPT

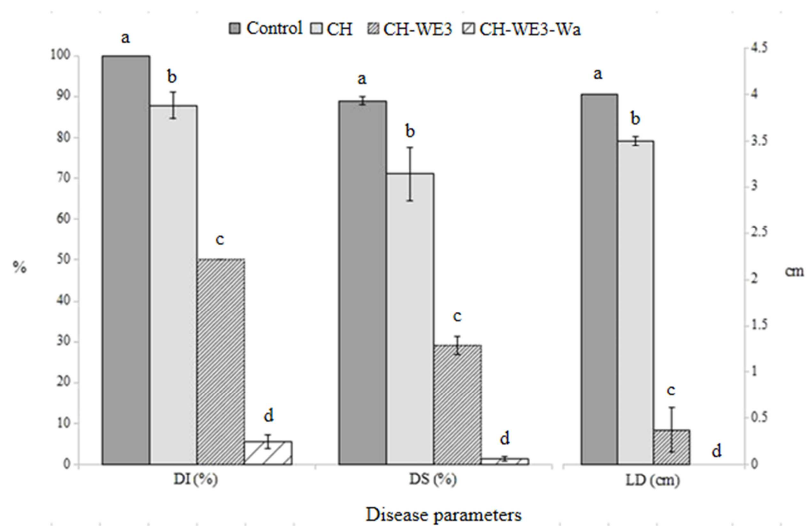


Fig. 1

ACCEPTED MANUSCRIPT

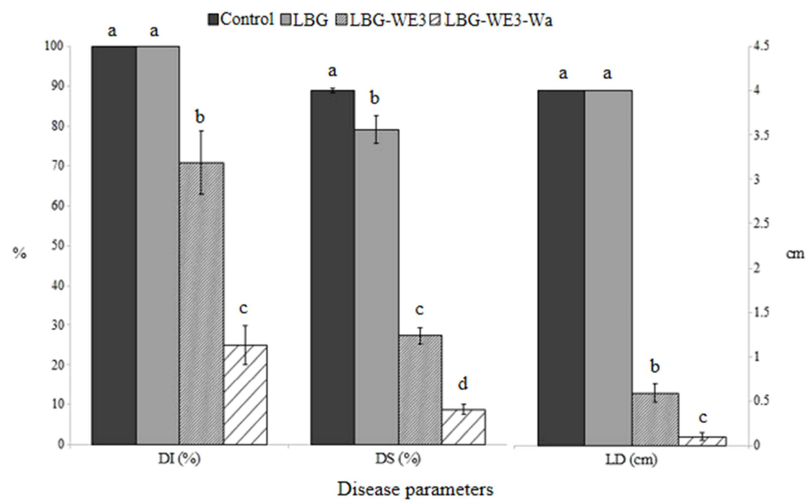


Fig. 2

ACCEPTED MANUSCRIPT

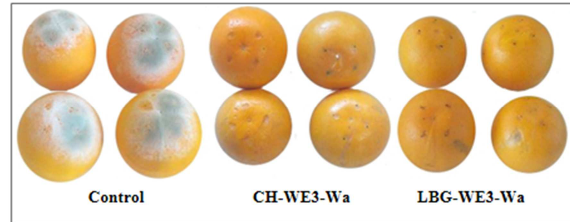


Fig. 3

ACCEPTED MANUSCRIPT

Highlights

ACCEPTED MANUSCRIPT

- Coatings carrying standardized pomegranate peel extract (PPE) inhibited *P. digitatum*
- Bioactive coatings reduced decay parameters on oranges inoculated with *P. digitatum*
- Biocontrol yeast *Wickerhamomyces anomalus* proved to be tolerant to PPE
- Coatings combining PPE with *W. anomalus* cells showed enhanced biocontrol efficacy

ACCEPTED MANUSCRIPT