Food Microbiology 74 (2018) 107–112

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

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

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ARTICLE INFO

*Article history:*
Available online 20 March 2018

*Keywords:*
Locust bean gum
Chitosan
*Wickerhamomyces anomalus*
Disease parameters

ABSTRACT

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

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



## 1. Introduction

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

The investigation of new eco-friendly approaches, such as the application of antagonistic microorganisms (Liu et al., 2013; Panebianco et al., 2015; Restuccia et al., 2006; Ruiz-Moyano et al., 2016; Scuderi et al., 2009) and/or of natural antimicrobial substances (Aloui et al., 2014; Palou et al., 2016) to control the postharvest mold decay, is preferred to synthetic fungicides to prevent negative or adverse effects on human health and nature balance. Such alternative solutions are considered relatively safe due to their

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https://doi.org/10.1016/j.fm.2018.03.011
0740-0020/

natural origin, biodegradability and low toxicity to the environment (da Cruz Cabral et al., 2013). In particular, a biological control which relies on antagonistic yeasts has been reported to be effective at managing postharvest decay of a variety of fruit (Liu et al., 2013). Many yeasts belonging to the genera *Candida*, *Metschnikowia*, *Wickerhamomyces* and *Aureobasidium* have been reported as effective biocontrol agents (BCAs) on postharvest diseases of citrus, apple, pear, grapefruit, table grape and sweet cherry (Aloui et al., 2015; Lutz et al., 2013; Oro et al., 2014; Parafati et al., 2015; Platania et al., 2012). In the domain of plant-derived compounds with antimicrobial potential, PPE has been extensively investigated for its free radical scavenging effect and strong antioxidant capacity caused by the high concentration of biologically active components, such as punicalagin, ellagic, gallic and chlorogenic acids (Elsherbiny et al., 2016; Kazemi et al., 2016; Kharchoufi et al., 2018). Few researches investigated extracts from pomegranate peel as natural inhibitors for plant pathogenic bacteria and fungi (Elsherbiny et al., 2016; Endo et al., 2010; Romeo et al., 2015), including *Penicillium digitatum* (Kharchoufi et al., 2018; Li Destri Nicosia et al., 2016). However, there are still major obstacles to the large-scale use of plant extracts to control postharvest pathogens. Although plant extracts have proved to be good antimicrobial agents, their use for maintaining fruit quality and reducing fungal decay is often limited by application costs, reduced and inconsistent efficacy as a result of fruit physiology and environment, low residual activity, lack of curative effect and limited range of activity against different fungal pathogens (Bautista-Banos et al., 2013).

The incorporation of these natural compounds into edible coating formulations can be an effective approach to solve some of these problems while, at the same time, controlling fruit postharvest decay by lowering the diffusion processes and maintaining high concentrations of active molecules at the fruit surface. Edible coatings could be considered a safer alternative solution for citrus fruit than wax coatings (Parafati et al., 2016), which usually are composed of oxidized polyethylene, organic solvents, surfactants and preservatives such as sodium methylparaben (Moscoso-Ramírez et al., 2013). Edible films and coatings, mainly constituted by starch, cellulose derivatives, chitosan/chitin, gums, proteins (animal or vegetable) and lipids, have been developed as natural or nonpolluting materials to replace commonly used waxes to extend the shelf-life of fruit, to improve fruit appearance, to reduce moisture losses, and eventually to incorporate antimicrobial food additives (Aloui et al., 2014; Valencia-Chamorro et al., 2011; Zhang et al., 2016a; b). Chitosan coatings have been widely reported to limit fungal decay and to delay the ripening of several commodities, including dates (Aloui et al., 2014), table grape (Guerra et al., 2016), and citrus fruit (Panebianco et al., 2014). Moreover, chitosan-based coatings can be used as a carrier to incorporate functional ingredients, such as antimicrobials, antioxidant enzymes, minerals, vitamins, and antioxidants. Locust bean gum (LBG) is a polysaccharide widely used in the production of edible films/coatings due to its edibility, biodegradability and hydrophilic properties (Barak and Mudgil, 2014; Sébastien et al., 2014), when dissolved in water solutions. LBG in edible films and coatings may also act as carrier of additives, bioactive compounds (Aloui et al., 2014) and biocontrol agents (Aloui et al., 2015; Parafati et al., 2016) whose viability over time can be accordingly extended.

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

## 2. Materials and methods

### 2.1. Microorganisms and culture conditions

*Penicillium digitatum* and *Wickerhamomyces anomalus* BS91 strains belong to the Di3A (Dipartimento di Agricoltura, Alimentazione e Ambiente, University of Catania, Italy) collection. *W. anomalus* was previously selected for its good antagonistic ability related to β-glucanase production (Muccilli et al., 2013; Parafati et al., 2016). More recently, bioactive coatings enriched with *W. anomalus* BS91, exhibited a good control of *P. digitatum* on mandarin and orange (Aloui et al., 2015; Parafati et al., 2016).

The mold and yeast stock cultures were stored at 4 °C on Petri dishes containing, respectively, Potato Dextrose Agar (PDA, CM0139, Oxoid, Basingstoke, UK) and Yeast Extract Peptone Dextrose Agar [YPDA: yeast extract, 10 g; peptone, 10 g; dextrose, 20 g; agar, 20 g (Oxoid, Basingstoke, UK) per liter of sterile distilled water (SDW)].

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

The chitosan (CH) film forming solution was prepared by dissolving CH (1%, w/v) in an aqueous glacial acetic acid solution (1%, v/v), at 40 °C for 12 h. The Locust bean gum (LBG) film forming solution was prepared by dissolving the LBG powder (molecular weight ~ 310,000 Da, Sigma Aldrich, Steinheim, Germany) in distilled water (0.5%, w/v) at 70 °C under constant agitation. Twenty % (w/w, based on biopolymer content) of glycerol (≥99% purity; Sigma-Aldrich, Steinheim, Germany) was used as a plasticizer to enhance the film flexibility and facilitate its release from the Petri plate.

The CH and LBG film forming solutions were modified by adding different amounts of either a water pomegranate peel extract (WPPE) or a methanol pomegranate peel extract (MPPE) (see Table 1), which had been prepared according to the procedure reported by Kharchoufi et al. (2018).

### 2.3. In vitro antifungal activity of active films carrying PPE

Ten mL of each active film forming solution (Table 1) was poured into Petri plates, and dried at room temperature for approximately 48 h to produce films with a controlled thickness. The films were then sterilely peeled off the Petri plates and preconditioned in climatic chamber at 25 °C and 75% RH, prior to testing.

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

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

### 2.4. W. anomalus tolerance to active films carrying PPE

The potential application of BCA *W. anomalus* to active films containing PPEs has been evaluated through a preliminary test. A disk of each dried active film (4 mm diameter), including either WPPE or MPPE, as described in Table 1, was cut and placed on a YPDA medium which had been previously inoculated according to the pour plate method with a 48-h cell suspension of *W. anomalus*

**Table 1**
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 |

(final concentration $10^6$ cells/mL), to test the influence of the active films on the growth of *W.* anomalus. The plates were incubated at 25 °C for 72 h.

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

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

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

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

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

### 2.6. Statistical analysis

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

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

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

## 3. Results and discussion

### 3.1. In vitro evaluation of the antifungal effectiveness of active coatings

In the present study the use of bioactive CH and LBG coatings, enriched with MPPE and WPPE was evaluated by means of *in vitro* assays against *P. digitatum.* Among plant-derived compounds, PPE, sourced from a largely available industrial byproduct, has gained attention for its antioxidant and antimicrobial properties (Tehranifar et al., 2011). The extracts used in the study were characterized to determine the total phenol content and profile (Kharchoufi et al., 2018), and the results demonstrated the presence

**Table 2**
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* |

of punicalagin as major component, which is an ellagitannin known for its antifungal activity (Glazer et al., 2012; Romeo et al., 2015).

Data from the antifungal assays, performed on PDA plates, showed that bioactive CH and LBG coatings, enriched with PPEs, significantly inhibited the growth of *P. digitatum* by producing an inhibition halo around the experimental bioactive film disks, which got larger at increasing WPPE or MPPE concentrations. Overall, the highest effectiveness was obtained using CH-ME3 and CH-WE3, which produced the widest inhibition zones (Table 3). Among the LBG coatings, only LBG-ME3 or −WE3 and LBG-ME2 or −WE2 produced significant inhibition halos on PDA inoculated with a *P. digitatum* conidial suspension, while no inhibition was observed in the control as well as in the formulation with the lowest PPE concentration (Table 3). These results are consistent with previous reports on the *in vitro* antifungal effect of PPE (Glazer et al., 2012; Kharchoufi et al., 2018; Osorio et al., 2010). It should be noted that neither LBG nor CH alone produced inhibition halos around the film sample; however, the growth of the mycelium was not observed in the contact area between CH and the agar medium, proving some intrinsic inhibitory activity of chitosan.

This effect was not observed for LBG alone, which, in fact, allowed the mycelium growth in the area of contact with the inoculated medium.

### 3.2. Influence of bioactive coatings on W. anomalus growth

With the aim to develop an integrated biological control approach to effectively manage postharvest *P. digitatum* decay *in vivo*, the opportunity to add a proved BCA, *W. anomalus* BS91, to the bioactive WPPE- and MPPE-coatings was evaluated by a preliminary *in vitro* assay. Such preliminary screening excluded any significant inhibition activity of the bioactive coatings, which caused weak growth inhibition (halo of 2 mm), only at the highest MPPE (0.304 g dry extract/mL) concentration. WPPE at all concentrations and MPPE at lower concentrations did not produce any appreciable inhibition effect against *W. anomalus* cells, nor did CH and LBG alone (no inhibition halo). Only few data regarding the effect of PPE against yeasts are available. The combination of punicalagin, identified as the main active antifungal compound of an hydro alcoholic extract prepared from the peel of *Punica granatum*, with fluconazole produced a potently synergistic action, by inducing ultrastructural changes against *Candida albicans* cells *in vitro* (Endo et al., 2010). The antifungal effects of pomegranate pericarp and peel extracts, attributed to changes in cell morphology and structure, were demonstrated against yeasts of the *Candida* genus (Anibal et al., 2013). Moreover, the crude extract of pomegranate peel showed activity against the dermatophytes *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, and *Microsporum gypseum*, with Minimum Inhibitory Concentrations (MICs) values of 125 μg/mL and 250 μg/mL, respectively for each genus (Foss et al., 2014). With regard to the effect of PPEs on food-related yeasts, the results obtained in this study are in agreement with those recently reported on *S. cerevisiae* (Kharchoufi et al., 2018). A possible explanation for this behavior could be attributed to the isolation source of *W. anomalus* BS91, the olive brine, which is particularly rich in plant polyphenols: this condition might have determined some adaptation of the BCA *W. anomalus* to such compounds. Moreover, additional experimental evidence proved that plant-derived bioactive compounds can extend the lifespan and improve resistance to oxidative stress in *Saccharomyces cerevisiae* (Martorell et al., 2011).

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

The *in vitro* assays demonstrated the effectiveness of CH and LBG coatings containing WPPE and MPPE against *P. digitatum* and excluded any interference on the growth of *W. anomalus*. On the basis of such results and in consideration of the fact that food-grade and environmentally-sustainable extraction methods from plant materials should be preferred, especially for their use as food supplement/additive (Kharchoufi et al., 2018), water pomegranate peel extract (WPPE) was selected for the subsequent *in vivo* trials on artificially pathogen-inoculated oranges.

The results of the biocontrol activity of CH and LBG-active coatings on oranges, which had been artificially inoculated with *P. digitatum*, are reported in Fig. 1 and Fig. 2, respectively. Both CH and LBG coatings incorporating 0.361 g dry WPPE/mL and *W. anomalus* cells significantly reduced green mold decay parameters (DI, DS and LD) on oranges, providing always significantly lower values if compared to the relative controls (Figs. 1 and 2). Based on the widely reported role of phenolic compounds as plant defense response inducer (Oliveira et al., 2016), it is likely that the postharvest application of PPE on fruit could induce mechanisms of resistance against fungal pathogens (Li Destri Nicosia et al., 2016).

Among chitosan coatings (Fig. 1), CH-WE3-Wa displayed the highest *in vivo* effectiveness at reducing DI, DS and LD values on wounded oranges (95, 98 and 100% of reduction, respectively, compared to the relative control), followed by the CH-WE3 treatment (49, 65 and 92% of reduction, respectively, compared to the relative control). The coating treatment with CH only determined an inferior reduction in the green mold decay parameters (10, 16 and 11% of reduction, respectively, compared to the relative control), as previously reported in literature (Panebianco et al., 2014, 2016). This effect could be due to the morphological and structural modifications induced by chitosan on fungal hyphae (Singh et al., 2008) and/or to the elicitation of biochemical defense responses in coated fruit (El Guilli et al., 2016).

Similarly, among the essayed LBG coatings (Fig. 2) the highest effectiveness in reducing DI, DS and LD values on wounded oranges was observed for the LBG-WE3-Wa combination (75, 92 and 98% of reduction, respectively, compared to the relative control), followed

**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 |

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

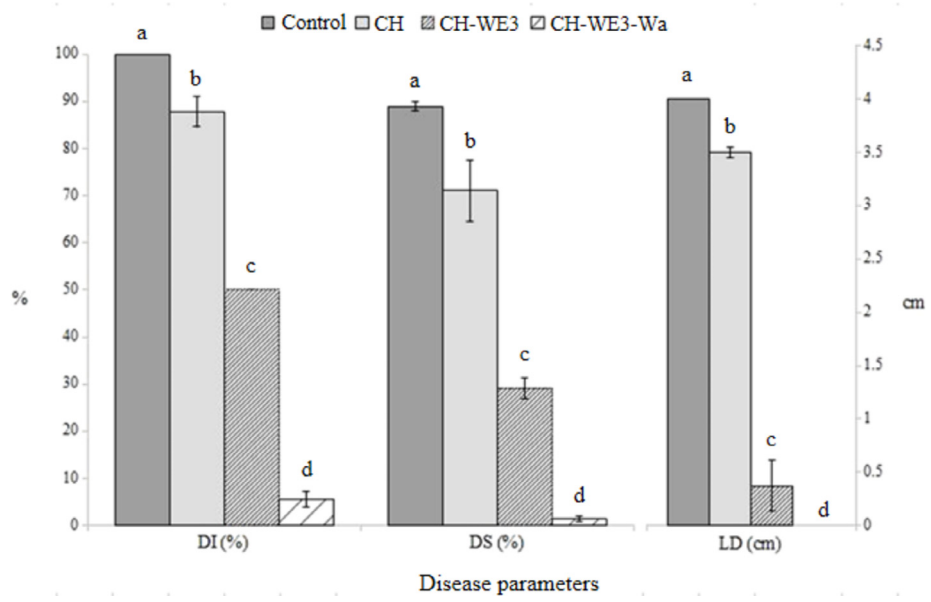


**Fig. 1.** Biocontrol effectiveness of chitosan coating incorporating 0.361 g dry WPPE/mL, alone or in combination with *W. anomalus* cells, against *Penicillium digitatum* on oranges. For treatments codification, please refer to Table 2. Bars indicate standard error of the mean. Columns within each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

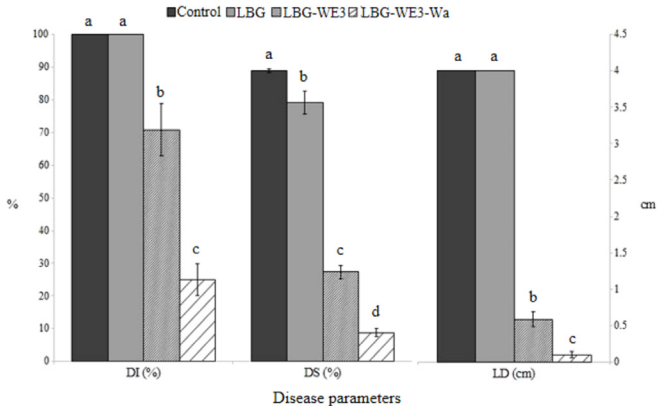


**Fig. 2.** Biocontrol effectiveness of LBG coating incorporating 0.361 g dry WPPE/mL, alone or in combination with *W. anomalus* cells, against *Penicillium digitatum* on oranges. For treatments codification, please refer to Table 2. Bars indicate standard error of the mean. Columns within each disease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


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

by the LBG-WE3 treatment (28, 69 and 85% of reduction, respectively, compared to the relative control). Orange coating comprising only LBG did not determine any appreciable reduction in the green mold decay in comparison with the relative control.

Overall, the highest reductions of the green mold diseases parameters were obtained by using CH-WE3-Wa followed by LBG-WE3-Wa (Fig. 3), which almost completely inhibited rot development on oranges.

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

## 4. Conclusions

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

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

extracted from pomegranate peel could replace potentially harmful synthetic preservatives. Moreover, the specific tolerance of biocontrol yeast *W. anomalus* BS91 to PPE, opens interesting strategies in the integrated management of postharvest decay and foreseeing advances in the development of new formulations which leverage on the synergy between biocontrol agents and natural bioactive compounds.

## Aknowledgements

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

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