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Effect of different coating-forming agents on the efficacy of the biocontrol agent *Candida sake* CPA-1 for control of *Botrytis cinerea* on grapes

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ABSTRACT

Multiple formulations of known biocontrol agent (BCA) *Candida sake*, containing different coating-forming polymers and surfactants were tested at different polymer:BCA ratios, in order to improve control of *Botrytis cinerea* on grapes. The BCA cell viability on the grape surface was analyzed and reduction in disease incidence and severity was determined. Coating-forming solids improved the survival and efficacy of *C. sake* as a BCA against *B. cinerea*, depending on the polymer type and ratio. The incorporation of surfactants did not improve survival or disease control, although they promoted a better cell dispersion on the grape surface. Cell growth of the antagonist during incubation led to the formation of aggregates, even when surfactants were present. Sodium caseinate and starch were the most suitable polymers to formulate *C. sake* preparations to obtain coating-forming systems with this BCA and to increase its survival and efficacy at the minimum economic cost of the ingredients.

Keywords: biological control, *Candida sake*, grapes, biopolymer, edible coating, microstructural analysis

1. INTRODUCTION

The fact that major post-harvest pathogens have developed resistance to many fungicides and the public demand for a reduction in pesticide use, stimulated by a greater awareness of environmental and health issues, have generated an increasing interest in alternative methods to fungicidal treatments in the control of fruit diseases (Teixidó *et al.*, 2011; Zahavi *et al*., 2000). Biological control, which consists of biologically-based processes to lower pathogen inoculum density and reduce crop loss, is one of the most effective and practical alternatives to chemical fungicides (Cañamás *et al*., 2011). Biocontrol has been extensively studied during the last twenty years; however, it is difficult to observe the successful results obtained under laboratory or controlled condition in pre-harvest conditions. Its commercial application has been greatly limited due to the narrow range of environmental conditions in which biocontrol agents (BCAs) are able to survive and effectively control pests and diseases. Hence, a main aim in the development and implementation of biological control products is to improve the ability of the antagonists to survive and successfully control postharvest diseases under a wider array of conditions and with minimal variability (Droby *et al*., 2003).

Several strategies have been employed to improve the behavior of BCAs in practical conditions. Physiological manipulation has been one of the strategies used to enhance the tolerance of BCAs to environmental stress conditions obtaining interesting results (Abadias *et al*., 2001; Liu *et al*., 2012; Mokiou and Magan, 2008; Teixidó *et al.*, 1998). Furthermore, diverse additives, such as coatings, can act as protectors during the preparation, conservation and application phases of antagonist-based products (Droby *et al*., 2009). These additives might maintain the viability of BCAs more effectively and promote their biocontrol efficacy. Moreover, additives could not only improve the spray

deposition, droplet size and spreadability of the products but also enhance survival and persistence of the BCAs under the stressing of conditions associated with the environmental fluxes in field. Cañamás *et al*., (2008a; 2008b) observed that the application of an edible coating improved the effectiveness of *Pantoea agglomerans* at controlling postharvest pathogens in orange fruit. Likewise, Cañamás *et al*., (2011) and Calvo-Garrido *et al*., (2013a; 2014b) demonstrated similar effect on *Candida sake* applied on grapes. This was attributed to the improvement in the environmental stress tolerance and ecological competence of this BCA. Other functions of coatings have been described so as to aid and enhance BCA survival, including protection from ultraviolet (UV) radiation, desiccation, rain and temperature variations and by acting as a source of nutrients. In addition, coatings may also slow the microbial desiccation, thereby extending the time available for the BCA to multiply and become established and improve their homogeneity and distribution on the plant surface (Cañamás *et al*., 2011). Therefore the combined application of BCAs and edible coatings offers many possibilities, both because of the wide variety of matrices which can be used and their potential benefits for the survival and retention of the antagonists.

Edible coatings, produced from biopolymers and food-grade additives, are thin layers of material that cover the surface of the food and can be consumed as a part of the whole product (Vargas *et al*., 2008). They have been widely studied for the purposes of maintaining the quality of coated products, mainly in post-harvest treatments of fruits and vegetables (Hernández-Muñoz *et al*., 2008; Pastor *et al*., 2011; Perdones *et al*., 2012). Thus, the pre-harvest application of edible coatings that incorporate a BCA could be a good strategy for the preservation of crops since them might enhance the activity of the antagonist and also provide benefits to fruit.

There is a wide spectrum of biopolymers (polysaccharides and proteins) that can be used as the main compounds in the obtaining of edible coatings. Among polysaccharides, hydroxypropylmethylcellulose (HPMC), is a remarkable coating-forming compounds and corn starch (S) is extensively used due to its low cost and high availability (Rodríguez *et al*., 2006). Likewise, dairy and plant proteins, such as sodium caseinate (NaCas) and pea protein (PP), are also coating-forming compounds of interest (Choi and Han, 2001; Sánchez-González *et al*., 2013). In order to enhance the wettability on the plant tissue and the adhesion of the coatings, it is good practice to incorporate surfactants to the biopolymer matrices as a means of decreasing the surface tension of the coating-forming dispersions (CFDs) (Krochta, 2002; Ortega-Toro *et al*., 2014). The balance between the polar and non-polar groups of the surfactant molecules determines their hydrophilic-lipophilic balance (HLB), which has a great influence on their surface activity depending on the blend components.

The enhancement of the efficacy of BCAs applied in combination with edible coating forming compounds has been demonstrated by several authors (Aloui *et al*., 2015; El Ghaouth *et al*., 2000; Fan *et al*., 2009; McGuire and Dimitriglou, 1999; McGuire *et al*., 2000; Potjewijd *et al*., 1995). However, there are, as yet, few studies aimed at the joint formulation of edible coatings and BCAs. Some isolated studies do exist, although a general overview is needed in order to acquire information about which is the most adequate design of edible coating in order to optimize the viability and effectiveness of the antagonists under practical conditions. The different physicochemical nature of the coating components can affect not only the viability and survival of the BCA but also their activity against the pathogen. It is important to consider the establishment of specific interactions between the polymeric matrix and the BCA and their influence on its biocontrol activity (Sánchez-González *et al*., 2013). Moreover, the type and

concentration of coating-forming solids in relation to the incorporated BCAs could change the environmental conditions and, consequently, affect their activity. Furthermore, these aspects could influence other features of key importance for practical application, such as the BCA adherence, or the thickness of the coating layer on the fruit surface.

The filamentous fungus *Botrytis cinerea* is the dominant bunch rot-causing pathogen of gray mold in grapes in many temperate regions of the world, producing significant crop losses (Elmer and Reglinski, 2006; Zahavi *et al*., 2000). Some studies have revealed that it is possible to protect grapes from gray mold disease using postharvest antifungal coatings (Romanazzi *et al*., 2007; Sánchez-González *et al*., 2011; Xu *et al*., 2007). Against grey mold, different yeasts have also exhibited antagonistic activity against *B. cinerea* (Elmer and Reglinski, 2006; Zahavi *et al*., 2000). Several studies have reported the efficacy of *Candida sake* CPA-1 yeast in controlling both gray mold in grapes (Calvo-Garrido *et al*., 2013a; 2013b; 2014b; Cañamás *et al*., 2011). In addition, it has been demonstrated that the use of the edible coating Fungicover® based on fatty acids allows to improve the efficacy of CPA-1 (Calvo-Garrido *et al.*, 2013a; 2014a).

Combining biocontrol and CFD agents in joint formulations to obtain active coatings could represent a good strategy to improve biocontrol efficacy. Hence, the aim of this work was to evaluate the effect of different CFDs containing *C. sake* CPA-1, based on different biopolymers (HPMC, S, NaCas or PP) with and without the addition of surfactants (oleic acid, OA, HLB: 1; Span 80, S80, HLB: 4.3; Tween 85, T85, HLB: 11), on the adherence, viability and survival of *C. sake* cells, as well as to test its biocontrol efficay against *B. cinerea* infections of coated grapes. The effect of coating-forming solids concentration respect to the BCA on these aspects was also analyzed for selected formulations. Likewise, scanning electron microscopy (SEM) was used to

analyze the distribution of *C. sake* on the surface of coated grapes for some formulations and times post application.

2. MATERIALS AND METHODS

2.1 Candida sake inoculum production

Strain CPA-1 of *Candida sake* (Viñas *et al.*, 1998) was originally isolated from the surface of apples by UdL-IRTA group (Lleida, Catalonia, Spain), and was deposited at the "Colección Española de Cultivos Tipo" (CECT-10817) in the "Universidad de Valencia" (Burjassot, Valencia, Spain). Cell production and formulation were carried out following methods described by Cañamás *et al*., (2011). Briefly, stock cultures were stored on nutrient yeast dextrose agar (NYDA) medium (nutrient broth, 8 g L^{-1}; dextrose 10 g L^{-1}; agar 15 g L^{-1}) at 4°C. When required, *C. sake* CPA-1 was sub-cultured onto NYDA plates at 25°C. Then, sub-cultured cells were suspended on potassium phosphate buffer (KH_2PO_4 0.2 M, 70 mL; K_2HPO_4 0.2 M, 30 mL; deionized water 300 mL) were added as inoculum starter to 5 L of molasses-based medium (cane molasses 40 g L^{-1}; urea 1.2 g L^{-1}; water activity a_w = 0.996), with adjustment of the initial concentration to 1×10^6 CFU mL^{-1}. Cell pellets were obtained by centrifugation at 6831 g for 10 min at 10°C after 40 h of liquid fermentation in a BIOSTAT-A modular bioreactor (Braun Biotech International, Melsungen, Germany) at 25°C, 400 rpm agitation speed and 150 L h^{-1} aeration level. Re-suspended pellets were then formulated in an isotonic solution, with adjustment of the water potential with trehalose as described by Abadias *et al*. (2003).

2.2 Preparation of the coating-forming dispersions with *Candida sake*

HPMC and NaCas were supplied by Sigma-Aldrich (Madrid, Spain). S and PP were purchased from Roquette Laisa España, S.A. (Valencia, Spain). CFDs, with and without

surfactants, were prepared by dispersing the biopolymers (2% w/v) in deionized water. HPMC was heated to 80°C for 10 min and maintained under magnetic stirring at 25°C overnight. S was maintained under stirring at 95°C for 30 min to induce starch gelatinization. NaCas and PP were dispersed at 25°C for 2 h. After polymer dispersion, glycerol (Panreac Química, S.L.U, Barcelona, Spain) was incorporated as plasticizer in S, NaCas and PP CFDs at a hydrocolloid:glycerol mass ratio of 1:0.25. Surfactants (all supplied by Sigma-Aldrich, Madrid, Spain) were added at a hydrocolloid:surfactant mass ratio of 1:0.1. The hydrocolloid:glycerol and hydrocolloid:surfactant ratios were selected on the basis of previous studies (Jiménez *et al*., 2012; Sánchez-González *et al*., 2009; Sánchez-González *et al*., 2013). CFDs were homogenized using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany) at 13,600 rpm for 4 minutes and sterilized at 121°C for 15 min.

Treatment	**Treatment description**
CS	*Candida sake* in sterilized deionized water
HPMC HPMC-OA HPMC-S80 HPMC-T85	Coating forming dispersions based on hydroxypropylmethylcellulose (HPMC) without surfactants or with oleic acid (OA), Span 80 (S80) or Tween 85 (T85).
S S-OA S-S80 S-T85	Coating forming dispersions based on corn starch (S) without surfactants or with oleic acid (OA), Span 80 (S80) or Tween 85 (T85).
NaCas NaCas-OA NaCas-S80 NaCas-T85	Coating forming dispersions based on sodium caseinate (NaCas) without surfactants or with oleic acid (OA), Span 80 (S80) or Tween 85 (T85)..
PP PP-OA PP-S80 PP-T85	Coating forming dispersions based on pea protein (PP) without surfactants or with oleic acid (OA), Span 80 (S80) or Tween 85 (T85).

After cooling, *C. sake* was incorporated in CFD to a final yeast concentration of 5×10^7 CFU mL^{-1} (Calvo-Garrido *et al*., 2013a; Cañamás *et al*., 2011). The dispersions obtained were shaken for 15 minutes at 150 rpm in a rotatory shaker (Selecta, Abrera,

166 Barcelona, Spain) to achieve a homogeneous distribution of the microorganisms. As a
167 control, a dispersion of *C. sake* in sterilized deionized water (CS) was prepared at 5×10^7
168 CFU mL^{-1}. The seventeen considered treatments are summarized in Table 1.

169 ### *2.3 Botrytis cinerea* inoculum

170 An isolate of *Botrytis cinerea* obtained from infected grapes collected in a local
171 vineyard in Lleida was used in this study because it was the most virulent isolate from
172 IRTA collection. The isolate was grown on potato dextrose agar (PDA) for 15 days at
173 20°C with a daily 14 h photoperiod of near ultraviolet light and 10 h dark to induce
174 sporulation. Conidial suspensions were prepared by adding 10 mL of sterile distilled
175 water containing 0.01% (w/v) Tween 80 to *B. cinerea* cultures. Conidia were scraped
176 from the agar using a sterile loop, sonicated for 5 min to facilitate conidial dispersion,
177 and then adjusted to 1×10^4 conidia mL^{-1} (Cañamás *et al*., 2011).

178 ### *2.4* Population dynamics of *Candida sake* on grapes

179 Six replicates, consisting of five berries of table grapes (*Vitis vinifera* L., Red Globe
180 variety) homogeneous in size and shape, were used for the application of each
181 treatment. The berries were selected on the basis of their maturity stage and without
182 signs of mechanical damage or fungal decay. Each sample was placed separately on a
183 plastic grid and sprayed for 5 seconds with its corresponding treatment, including CS
184 control, using an air brush. The samples were left to dry at room temperature and then
185 placed in a sealed plastic box for incubation at 20°C and 85% RH for either 24 h or 7
186 days. To study the population dynamics, each sample was weighed and transferred to
187 Erlenmeyer flasks containing 100 mL of sterile deionized water with 0.01% (w/v)
188 Tween 80. They were shaken in a rotatory shaker at 150 rpm for 20 min and sonicated
189 for 10 min in an ultrasound bath (Selecta, Abrera, Barcelona, Spain) to achieve the

maximum detachment of the yeast from the grape surface. Serial dilutions of the washings were performed in duplicate and plated onto NYDA agar medium with streptomycin sulphate (Sigma-Aldrich, Madrid, Spain) at a concentration of 0.5 g L^{-1} to prevent bacterial growth. Plates were incubated for 48 h at 25°C in the dark and typical *C. sake* colonies were then counted based on their morphological characteristics. Results were expressed as log CFU per gram of treated grape.

2.5 Efficacy of *Candida sake* against *Botrytis cinerea* on grapes

Three replicates of five berries each per formulation were used to study the effectiveness of the antagonist in the biocontrol against *B. cinerea*. Samples were washed with water, left to dry and placed separately on plastic grids. Sandpaper was used to induce the rupture of the grape cuticle and favour pathogen infection. The different CFDs with *C. sake* were applied as described in section 2.4, as well as the CS control and an additional deionized water control without the antagonist (W). When the berries were dried, a conidial suspension of *B. cinerea* at 1×10^4 conidia mL^{-1} was applied with an air brush and left to dry again at room temperature. Samples were incubated at 20°C and 85% RH for either 7 or 12 days. Likewise, CFDs without the incorporation of the BCA were applied in order to ascertain if they exert any antifungal effect against de pathogen.

The incidence of the pathogen rot was visually evaluated by counting the number of berries with the typical *B. cinerea* conidia. The severity of the pathogen infection was visually estimated and expressed as the percentage of berry surface affected by grey mold (Cañamás *et al*., 2011). The results were expressed as the percentage reduction of the incidence and severity as referred to W.

2.6 Microstructural analysis of coatings containing *Candida sake* on grape surface

The microstructural analysis of grape surfaces coated with the formulations S, S-OA, S-T85, NaCas, NaCas-OA and NaCas-T85 containing 5×10^7 CFU mL^{-1} of *C. sake* was carried out after 24 h and 7 days post application by cryoSEM using a Scanning Electron Microscope (JEOL JSM-5410, Japan). Samples were cryofixed in slush nitrogen and observed, after gold coating, using an accelerating voltage of 10 kV. Images of the coated grape surface were obtained to analyze the distribution of *C. sake* on the grape surface with different coating formulations.

2.7 Influence of the ratio of coating-forming solids:BCA on *Candida sake* viability and efficacy

The influence of the ratio of the coating-forming solids with respect to the *C. sake* concentration in the CFDs on the antagonist efficacy was also analyzed for some selected formulations, in order to know if there is a critical ratio for promoting its viability and efficacy as BCA. In practical terms, it would be preferable a minimum amount of solids in order to limit the quantity of non-active material and to obtain a final product with a competitive price. To this end, two of the initially tested biopolymers were selected: NaCas, for its positive results and S due to its low cost. Some modifications of the initial formulations were introduced: native corn starch was replaced by pre-gelatinized corn starch to avoid the necessary gelatinization; and the use of glycerol as plasticizer was discarded since its incorporation into the CFDs did not have a positive effect on the activity of the antagonist, as deduced from a previous study (data not shown).

CFDs based on NaCas and S were prepared with different ratios of coating-forming solids maintaining a *C. sake* concentration of 5×10^7 CFU mL^{-1}. The concentrations of coating solids used were: 25, 12.5, 16.25, 5, 3.75, 2.5 or 1.25 mg mL^{-1}. The mass of the hydrocolloid required for each treatment was dispersed in 50 mL deionized water and

239 after its complete dispersion, the CFD were sterilized at 121°C for 15 min. *C. sake* was
240 incorporated into CFDs at the yeast concentration required as in section 2.2. The
241 adherence and survival of *C. sake* on grapes and its efficacy against *B. cinerea* for the
242 different CFDs were analyzed as described in sections 2.4 and 2.5. The results of the
243 population dynamics were expressed as the difference of log CFU per gram of treated
244 grape with respect to the CS treatment without coating solids. For the efficacy assays,
245 the percentage of incidence and severity reduction referred to the W was also reported.

246 ***2.8*** **Statistical analysis**

247 The statistical analysis of the population dynamics of *C. sake* and the incidence and
248 severity of *B. cinerea* infection was performed through an analysis of variance
249 (ANOVA) using Statgraphics Centurion XVI version 16.1.17 (Manugistics Corp.,
250 Rockville, Md.). CFU data were log-transformed prior to ANOVA to improve the
251 homogeneity of variances. Significant differences were determined using LSD test ($p <$
252 0.05).

3. RESULTS AND DISCUSSION

3.1 Population dynamics of *Candida sake* on grapes

The influence of the different CFD formulations on the population data of *C. sake* on grapes can be observed in Figure 1. 24 h after application, *C. sake* populations on berry surface were between 5 and 6 log CFU g^{-1} in every case, as previously reported by Cañamás *et al*. (2011) in field experiments using CPA-1. Significantly ($p < 0.05$) higher values, compared to the control treatment (CS), were observed for S, NaCas and PP-OA formulations. In this sense, a high rate of yeast survival after the application step is important to ensure that there is a high number of CFU available to colonize the fruit surface (McGuire and Dimitriglou, 1999). After 7 days of incubation, an increase in the *C. sake* population was observed for all treatments, including the one without coating-forming agents. All NaCas-based coatings, with and without surfactants, PP-OA and PP-T85 showed a significantly higher ($p < 0.05$) population on berries than the rest of treatments, including the control. These results indicate that all of the coating formulations used were suitable carriers for the microorganism and allowed *C.sake* cells to effectively establish on the fruit surface. It is likely that the layer created by these coatings on the berry surface could generate a beneficial environment for the BCA that would stimulate its survival (Cañamás *et al*., 2011). Other authors have reported beneficial effects of some components on the survival of some BCAs. McGuire and Baldwin (1994) and McGuire and Dimitriglou (1999) reported that coatings based on cellulose or sucrose esters supported high numbers of the yeast *Candida oleophila* when applied on grapefruits. Similarly, Potjewijd *et al*. (1995) observed that a methylcellulose-based coating applied on oranges was the best carrier for the pathogen antagonists *Candida guillermondii* and *Debaromyces spp*.

277 In general, all protein-based coatings (NaCas and PP) led to the highest initial adherence
278 and survival rate of the yeast, promoting their growth during incubation time, which
279 was especially notable for formulations with NaCas. In these formulations, the highest
280 value of log CFU g^{-1}, 6.89, was observed with no significant effect of surfactants. In
281 general, surfactants did not significantly affect ($p > 0.05$) the BCA survival, except for
282 PP formulations, where the addition of OA and T85 had a marked positive effect after 7
283 incubation days. The positive effect of proteins could be attributed to a better
284 availability of adequate nutrients for *C. sake*. In the case of polysaccharides, S showed a
285 significantly higher population of cells with respect to CS, but only after 24 h of
286 incubation.

287 The presence of a high number of CFU available to rapidly germinate and grow on the
288 fruit surface before the arrival of the pathogen is a key factor in the prevention or
289 reduction of the disease development, especially when the mechanism of action is based
290 on the competition for space and nutrients, such as it is described for *C. sake* (Fokkema,
291 1996; Ippolito and Nigro, 2000). In this sense, formulations with NaCas and S would be
292 suitable to improve the biocontrol effect of the yeast.

293 ### *3.2* Efficacy of *Candida sake* against *Botrytis cinerea* on grapes

294 The effect of the different formulations on the effectiveness of *C. sake* in the biocontrol
295 of *B. cinerea* is shown in Figure 2, as the percentage of incidence reduction with respect
296 to the control sample W, after 7 and 12 days of inoculation. The untreated controls
297 showed an incidence infection of 86% and 96.5% after 7 and 12 days of infection,
298 respectively, and a value of infection severity of 70.5%. In general, all treatments
299 exhibited a similar or higher reduction than the CS control after 7 days of incubation.
300 Several treatments showed a significantly ($p < 0.05$) higher reduction of the infection

with respect to the solid-free formulation of *C. sake*. The highest reduction was obtained for the S-T85, HPMC-S80 and PP-OA treatments, with reduction values higher than 80%. PP, HPMC, NaCas and NaCas-OA treatments also reached good levels of biocontrol but the reductions were slightly lower. In agreement with our results El Ghaouth *et al*. (2000) reported that the combination of the yeast *Candida satoiana* with glycolchitosan was more effective controlling gray mold on apple caused by *B. cinerea* than the independent applications of *C. saitoana*.

After 12 days of incubation, the reduction of the incidence decreased in every case because of the progression of the existing infection. Nevertheless, some of the applied treatments (S-T85, NaCas, NaCas-OA, PP and PP-OA) still maintained a significantly higher reduction of the *Botrytis* incidence than CS control. Among the treatments which better controlled the pathogen growth at 7 days, those containing HPMC were not effective after 12 days of incubation, which could be associated with the lack of yeast viability after long times in this substrate. The infection control with both proteins and S coatings was coherent with the greatest viability of *C. sake* in these supports, as previously mentioned.

The effectiveness of the CFDs without BCA was also evaluated (data not shown) and no significant effect could be observed in the control of the infection, since all formulations showed low or no effect. This indicates that components used in the CFDs did not themselves exhibit antifungal effects, although they could enhance the BCA action through different mechanisms, such as supplying adequate nutrients or water retention contribution.

Nevertheless, several authors have found effectiveness against some pathogens of some coatings. For example, Calvo-Garrido *et al*. (2014a) demonstrated the efficacy of a fatty

acid-based product with coating-forming ability against *B. cinerea* by a multiple mode of action. Other studies have reported the use of fatty acid-based products in other fruit crops to act against *B. cinerea* and other fungal pathogens (Hou and Forman 2000; Montealegre *et al*., 2010; Řiháková *et al*., 2001). Likewise, chitosan-based coatings have been widely studied due to its antimicrobial properties (Reglinski *et al*., 2010; Romanazzi *et al*., 2009) which can be promoted by the incorporation of other bioactive compounds (Sánchez-González *et al*., 2011; Perdones *et al*., 2012). Aloui *et al*., (2014; 2015) also reported that sodium alginate and locust bean gum based coatings had a slight indirect effect on the fungal decay of oranges and grapes.

The effect of surfactants on the infection control was not related to their effect observed on the viability of yeast cells. For NaCas, the same cell viability was obtained for treatments with and without surfactants, whereas the incidence reduction by formulations was only notable in surfactant-free samples or those with OA. In the case of PP treatments, T85 enhanced yeast viability but did not improve the infection control. On the contrary, T85, which was not effective at promoting *C. sake* viability in S formulations, significantly improved its biocontrol efficacy. This suggests that the interactions of the support components, not only with the BCA but also with the infectious agent, play an important role in biocontrol.

All protein-based coatings showed a better control of the severity of the infection. NaCas, NaCas-OA, NaCas-T85, PP and PP-OA significantly improved ($p < 0.05$) the reduction of infection severity with respect to CS treatment. S-T85 treatments achieved levels of control similar to those of the mentioned CFDs based on proteins. These results suggest that the overall balance of interactions among molecular components of CFD and the antagonist cells affected the final action of the BCA against the pathogen. In fact, McGuire and Hagenmaier (1996) reported a presumable effect of some

compounds of commercial coatings, such as surfactants, on microbial survival, including that of pathogens.

3.3 Microstructural analysis of coatings containing C*andida sake* on grape surface

In order to analyze the distribution of cells on the grape surface as well as their possible morphological changes throughout time, SEM observations were carried out on newly NaCas and S coated samples (24h after coating treatment) and on those stored for 7 days at 20°C and 85% RH . The same samples, with the addition of OA and T85 surfactants, were also observed in order to analyze the effect of these surfactants on the cell distribution on the grape surface. Figure 4 shows representative images of the surface of grapes coated with *C. sake* dispersions in water (CS) and in the CFDs based on NaCas and S. The grape surfaces were partially covered by the coatings and the BCA cells were surrounded by a biofilm (Figure 4b), which was probably excreted for their protection. In grapes with CS, the typical crystalline formations of the epicuticular natural surface wax were observed (Fava *et al*., 2011). This waxy structure appeared coated with a polymer layer when bioactive coatings incorporating *C. sake* were applied on grape surface. Grape surface appeared smoother, more homogeneous, and more uniform as a result of coatings application. In general, the coating distribution was uneven since coated and uncoated areas were observed in the samples. The surface coated with NaCas formulations (Figures 4c and 4d) exhibited a more granular appearance due to the globular structure of the protein. In contrast, S based coatings (Figures 4e and 4f) led to a smoother and more homogeneous surface.

The formation of cell aggregates was observed, probably as result of the natural tendency of microorganisms to attach onto solid surfaces thereby forming biofilms (Domínguez-Manzano *et al.*, 2012), while it could be also promoted by the loss of water

374 during the coating drying. Biofilm formation includes the bonding of the cells to a solid
375 surface and the presence of an extracellular matrix (Nobile and Mitchell, 2007). Cell
376 aggregates were more extensive and multilayered in water-coated grapes, whereas the
377 presence of coatings resulted in monolayer accumulations.

378 In grapes treated with NaCas coatings (Figures 4c and 4d), the cells appeared more
379 irregularly coated and small globular protein particles were observed on their surface.
380 On the contrary, a greater coverage was observed in grapes treated with S (Figures 4e
381 and 4f). This could be explained by the ability of the polysaccharide chains to coat the
382 cells and fill the gaps between them.

383 In Figures 5 and 6, the effect of the incorporation of the surfactant on the formulation of
384 NaCas and S coatings can be observed after 1 and 7 days of application. Surfactants
385 induced a greater disaggregation of cells, which appeared much more dispersed and
386 isolated on the surface as compared to grapes coated with both polymers without
387 surfactants. So, the incorporation of surfactants reduced the formation of aggregates.
388 Likewise, the appearance of the coatings was more heterogeneous due to the lack of
389 miscibility of the surfactants with the polymers, which gave rise to lipid dispersed
390 particles inside the polymer matrix, depending on the polymer-surfactant interactions.
391 The observed heterogeneity of the coatings with surfactants has been previously
392 described also from SEM micrographs of starch-surfactant based films by Jiménez *et*
393 *al*., (2012) and Ortega-Toro *et al*. (2014). After 7 days of incubation, clusters of cells
394 were again observed on the grape surface treated with S and NaCas with surfactants,
395 which might be attributed to the yeast growth from the initial isolated cells with the
396 subsequent increase in their population, as previously commented on. In the case of
397 grapes coated with S and surfactants, some *C. sake* cells exhibited a more elongated
398 appearance probably associated to their division process (Figures 6b and 6d). In grapes

399 coated with NaCas and surfactants the aggregates showed different layers (Figures 5b
400 and 5d). The cells in the layers below presented a dehydrated aspect as compared to
401 cells in the upper part. The appearance observed for the new cells in NaCas and S films
402 was different. Cells in S treated samples became more dehydrated and were less vital in
403 appearance than those coated with NaCas. Thus, SEM images revealed an apparently
404 better preservation and vitality of *C. sake* when NaCas was used in BCA formulation.
405 This agrees with the higher counts obtained for NaCas treated samples after 7
406 incubation days.

407 ### *3.4* Influence of the ratio of coating-forming solids:BCA on *Candida sake* viability
408 and efficacy

409 The effect of the proportion of coating-forming solids with respect to the concentration
410 of BCA was analyzed in order to establish the minimum amount of solids that improve
411 the antagonistic activity. For this purpose, coatings based on NaCas and S were
412 selected, as explained above. Data were analyzed in terms of the relative increase in the
413 BCA population (Δlog CFU) with respect to the corresponding control (CS) after the
414 different incubation times (24 h and 7 days; figure 7). It is remarkable that the coatings
415 had positive (+Δlog CFU) and negative effects (-Δlog CFU) on the population of *C.*
416 *sake*, depending on the incubation time, solid ratios and polymer type. In general, after
417 24 h, coatings based on S had a positive effect over the whole range of solid ratios,
418 while those based on NaCas only had a positive effect when applied at low
419 concentrations (2.5, 3.75 and 5 mg mL^{-1}). The behavior of NaCas-based coatings with
420 low solid concentractions was contrary to the observed tendencies (lines in the plot),
421 whereby the higher the solid ratio the higher the Δlog increase. Concerning the
422 incubation time, a negative effect was always observed in coatings based on S, whereas
423 for NaCas-based coatings a positive effect was found but only for the highest

concentrations (upper 5 mg mL^{-1}) where lower counts were obtained after 24 h of incubation. For a high ratio of solids:BCA, these results agreed with those observed in the first experimental series carried out with a solid concentration of 20 mg mL^{-1}. As previously commented on, a population increase in *C. sake* was observed for NaCas coatings, whereas no significant cell growth occurred in S coated samples during the 7 incubation days. Likewise, the SEM micrographs also showed the *C. sake* growth in NaCas coated grapes during 7 incubation days, whereas although cells in S coated grapes seemed to grow, they appeared altered in shape in the micrographs.

This behaviour suggests that in order to ensure the better survival of *C. sake* during the coating drying and incubation time, a minimum concentration of coating solids is required, although this value is dependent on the kind of solids. NaCas better preserved the viability of *C. sake* during incubation time, promoting its growth; and the greater the solid ratio was, the higher the cell count difference with respect to the control after 7 incubation days. Although this same tendency was observed for S coatings, the colony number significantly decreased after 7 incubation days.

The effect of the coating solid ratio on the reduction of the incidence and severity of the *B. cinerea* infection was also analyzed after 6 days of incubation (Figures 8 and 9). A significantly greater incidence reduction was observed for high solid ratios. The treatments with a significant reduction in the incidence with respect to the CS treatment were those containing more than 5 mg mL^{-1} of NaCas or more than 2.5 mg mL^{-1} of S (except intermediate values, 5 and 6.5 mg mL^{-1} for S, where no significant differences were found). Similarly, the reduction in the severity of the infection (Figure 9) was significantly higher than that of the CS treatment when the NaCas concentration was higher than 5 mg mL^{-1} and when the S concentration was 2.5, 3.75 or 25 mg mL^{-1}. Therefore, the amount of coating solids in relation to the CFU had an effect on the

449 efficacy of *C. sake* against *B. cinerea*, which was also dependent on the kind of
450 polymer. The use of NaCas gave rise to a good efficacy of the BCA at a higher solid
451 ratio than S, in line with its better support for the growth of the *C. sake* during
452 incubation time. The improvement in the efficacy of *C. sake* at controlling *B. cinerea*
453 agreed with the increase in the population of the BCA throughout time and the vital
454 appearance of the cells in SEM micrographs, which guarantees their biocontrol action.
455 This was confirmed in the second experimental series with different ratios of coating-
456 forming solids with respect to the BCA CFUs. After 7 incubation days, greater cell
457 counts could be observed for NaCas than for S coatings, both of which were higher
458 when the coating-forming solids increased in the formulation. The greater nutrient
459 availability for cells on the grape surface and the better limitation of cell drying
460 throughout time, when a high ratio of coating-forming solids covered the grapes, could
461 explain this finding.

462 **4. CONCLUSIONS**

463 In conclusion, coating-forming solids improved the survival and efficacy of *C. sake* as
464 BCA of *B. cinerea*, depending on the polymer type and ratio of coating solids. The
465 addition of surfactants did not imply additional positive effects, although they promoted
466 a better cell dispersion onto the grape surface. Nevertheless, cell growth during the
467 incubation time led to the formation of cell aggregates, even when surfactants were
468 added to the formulations. Taking into account the relative increase in the survival and
469 efficacy of *C. sake*, and the cost of ingredients, NaCas or S are recommended to
470 formulate preparations in order to obtain coating-forming systems with this BCA
471 against *B. cinerea* in grapes. The highest polymer:CFU ratios in the formulation
472 exhibited better biocontrol properties and so, this is also recommended. For NaCas, at
473 least 6 mg for 5×10^{7} CFU mL^{-1} was required to ensure the effective biocontrol of *B.*

474 *cinerea.* In the case of S, 2.5 mg for 5×10^7 mL^{-1} CFU also led to an improved effective
475 biocontrol.

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481 **6. REFERENCES**

482 Abadias, M., Teixidó, N., Usall, J., Viñas, I., Magan, N., 2001. Improving water stress
483 tolerance of the biocontrol yeast *Candida sake* grown in molasses-based media by
484 physiological manipulation. Can. J. Microbiol. 47, 123–129. doi:10.1139/w00-138

485 Abadias, M., Usall, J., Teixidó, N., Viñas, I., 2003. Liquid formulation of the
486 postharvest biocontrol agent *Candida sake* CPA-1 in isotonic solutions. Phytopathology
487 93, 436–442. doi:10.1094/PHYTO.2003.93.4.436

488 Aloui, H., Khwaldia, K., Sánchez-González, L., Muneret, L., Jeandel, C., Hamdi, M.,
489 Desobry, S., 2014. Alginate coatings containing grapefruit essential oil or grapefruit
490 seed extract for grapes preservation. Int. J. Food Sci. Technol. 49, 952–959.
491 doi:10.1111/ijfs.12387

492 Aloui, H., Licciardello, F., Khwaldia, K., Hamdi, M., Restuccia, C., 2015. Physical
493 properties and antifungal activity of bioactive films containing *Wickerhamomyces*
494 *anomalus* killer yeast and their application for preservation of oranges and control of
495 postharvest green mold caused by Penicillium digitatum. Int. J. Food Microbiol. 200,
496 22–30. doi:10.1016/j.ijfoodmicro.2015.01.015

Calvo-Garrido, C., Elmer, P. a G., Viñas, I., Usall, J., Bartra, E., Teixidó, N., 2013a. Biological control of botrytis bunch rot in organic wine grapes with the yeast antagonist *Candida sake* CPA-1. Plant Pathol. 62, 510–519. doi:10.1111/j.1365-3059.2012.02684.x

Calvo-Garrido, C., Viñas, I., Elmer, P., Usall, J., Teixidó, N., 2013b. *Candida sake* CPA-1 and other biologically based products as potential control strategies to reduce sour rot of grapes. Lett. Appl. Microbiol. 57, 356–361. doi:10.1111/lam.12121

Calvo-Garrido, C., Elmer, P. a G., Parry, F.J., Viñas, I., Usall, J., Torres, R., Agnew, R.H., Teixidó, N., 2014a. Mode of action of a fatty acid-based natural product to control *Botrytis cinerea* in grapes. J. Appl. Microbiol. 116, 967–979. doi:10.1111/jam.12430

Calvo-Garrido, C., Viñas, I., Usall, J., Rodríguez-Romera, M., Ramos, M.C., Teixidó, N., 2014b. Survival of the biological control agent *Candida sake* CPA-1 on grapes under the influence of abiotic factors. J. Appl. Microbiol. 117, 800–811. doi:10.1111/jam.12570

Cañamás, T.P., Viñas, I., Usall, J., Casals, C., Solsona, C., Teixidó, N., 2008a. Control of postharvest diseases on citrus fruit by preharvest application of the biocontrol agent *Pantoea agglomerans* CPA-2. Part I. Study of different formulation strategies to improve survival of cells in unfavourable environmental conditions. Postharvest Biol. Technol. 49, 86–95. doi:10.1016/j.postharvbio.2007.12.006

Cañamás, T.P., Viñas, I., Usall, J., Torres, R., Anguera, M., Teixidó, N., 2008b. Control of postharvest diseases on citrus fruit by preharvest applications of biocontrol agent *Pantoea agglomerans* CPA-2. Part II. Effectiveness of different cell formulations. Postharvest Biol. Technol. 49, 96–106. doi:10.1016/j.postharvbio.2007.12.005

520 Cañamás, T.P., Viñas, I., Torres, R., Usall, J., Solsona, C., Teixidó, N., 2011. Field
521 applications of improved formulations of *Candida sake* CPA-1 for control of *Botrytis*
522 *cinerea* in grapes. Biol. Control 56, 150–158. doi:10.1016/j.biocontrol.2010.11.007

523 Choi, W., Han, J.H., 2001. Physical and mechanical properties of pea-protein-based
524 edible films. J. Food Sci. 66, 319–322.

525 Domínguez-Manzano, J., Olmo-Ruiz, C., Bautista-Gallego, J., Arroyo-López, F.N.,
526 Garrido-Fernández, A., Jiménez-Díaz, R., 2012. Biofilm formation on abiotic and biotic
527 surfaces during Spanish style green table olive fermentation. Int. J. Food Microbiol.
528 157, 230–238. doi:10.1016/j.ijfoodmicro.2012.05.011

529 Droby, S., Wisniewski, M., El Ghaouth, A., Wilson, C., 2003. Influence of food
530 additives on the control of postharvest rots of apple and peach and efficacy of the yeast-
531 based biocontrol product Aspire. Postharvest Biol. Technol. 27, 127–135.
532 doi:10.1016/S0925-5214(02)00046-7

533 Droby, S., Wisniewski, M., Macarisin, D., Wilson, C., 2009. Twenty years of
534 postharvest biocontrol research: Is it time for a new paradigm? Postharvest Biol.
535 Technol. 52, 137–145. doi:10.1016/j.postharvbio.2008.11.009

536 El-Ghaouth, A., Smilanick, J.L., Wilson, C.L., 2000. Enhancement of the performance
537 of *Candida saitoana* by the addition of glycolchitosan for the control of postharvest
538 decay of apple and citrus fruit. Postharvest Biol. Technol. 19, 103–110.
539 doi:10.1016/S0925-5214(00)00076-4

540 Elmer, P.A.G., Reglinski, T., 2006. Biosuppression of *Botrytis cinerea* in grapes. Plant
541 Pathol. 55, 155–177. doi:10.1111/j.1365-3059.2006.01348.x

542 Fan, Y., Xu, Y., Wang, D., Zhang, L., Sun, J., Sun, L., Zhang, B., 2009. Effect of
543 alginate coating combined with yeast antagonist on strawberry (*Fragaria* × *ananassa*)
544 preservation quality. Postharvest Biol. Technol. 53, 84–90.
545 doi:10.1016/j.postharvbio.2009.03.002

546 Fava, J., Hodara, K., Nieto, A., Guerrero, S., Alzamora, S.M., Castro, M.A., 2011.
547 Structure (micro, ultra, nano), color and mechanical properties of *Vitis labrusca* L.
548 (grape berry) fruits treated by hydrogen peroxide, UV-C irradiation and ultrasound.
549 Food Res. Int. 44, 2938–2948. doi:10.1016/j.foodres.2011.06.053

550 Fokkema, N.J., 1996. Biological control of fungal plant diseases. Entomophaga 41,
551 333–342. doi:10.1007/BF02765788

552 Hernández-Muñoz, P., Almenar, E., Valle, V., Velez, D., Gavara, R., 2008. Effect of
553 chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria*
554 × *ananassa*) quality during refrigerated storage. Food Chem. 110, 428–435.
555 doi:10.1016/j.foodchem.2008.02.020

556 Hou, C.T., Forman III, R.J., 2000. Growth inhibition of plant pathogenic fungi by
557 hydroxy fatty acids. J. Ind. Microbiol. Biotechnol. 24, 275–276.

558 Ippolito, A., Nigro, F., 2000. Impact of preharvest application of biological control
559 agents on postharvest diseases of fresh fruits and vegetables. Crop Prot. 19, 715–723.
560 doi:10.1016/S0261-2194(00)00095-8

561 Jiménez, A., Fabra, M.J., Talens, P., Chiralt, A., 2012. Effect of re-crystallization on
562 tensile, optical and water vapour barrier properties of corn starch films containing fatty
563 acids. Food Hydrocoll. 26, 302–310. doi:10.1016/j.foodhyd.2011.06.009

564 Liu, J., Wisniewski, M., Droby, S., Norelli, J., Hershkovitz, V., Tian, S., Farrell, R.,
565 2012. Increase in antioxidant gene transcripts, stress tolerance and biocontrol efficacy of
566 *Candida oleophila* following sublethal oxidative stress exposure. FEMS Microbiol.
567 Ecol. 80, 578–590. doi:10.1111/j.1574-6941.2012.01324.x

568 McGuire, R.G., 2000. Population dynamics of postharvest decay antagonists growing
569 epiphytically and within wounds on grapefruit. Phytopathology 90, 1217–1223.
570 doi:10.1094/PHYTO.2000.90.11.1217

571 Mcguire, R.G., Baldwin, E.A., 1994. Compositions of cellulose coatings affect
572 populations of yeasts in the liquid formulation and on coated grapefruits. Proc. Florida
573 State Hortic. Soc. 107, 293–297.

574 McGuire, R.G., Dimitroglou, D.A., 1999. Evaluation of shellac and sucrose ester fruit
575 coating formulations that support biological control of post-harvest grapefruit decay.
576 Biocontrol Sci. Technol. 9, 53–65. doi:10.1080/09583159929901

577 McGuire, R.G., Hagenmaier, R.D., 1996. Shellac coatings for grapefruits that favor
578 biological control of *Penicillium digitatum* by *Candida oleophila*. Biol. Control 7, 100–
579 106. doi:10.1006/bcon.1996.0071

580 Mokiou, S., Magan, N., 2008. Physiological manipulation and formulation of the
581 biocontrol yeast *Pichia anomala* for control of *Penicillium verrucosum* and ochratoxin
582 A contamination of moist grain. Biocontrol Sci. Technol. 18, 1063–1073.
583 doi:10.1080/09583150802585769

584 Montealegre, J.R., López, C., Stadnik, M.J., Henríquez, J.L., Herrera, R., Polanco, R.,
585 Di Piero, R.. M., Pérez, L.M., 2010. Control of grey rot of apple fruits by biologically

586 active natural products. Trop. Plant Pathol. 35, 271–276. doi:10.1590/S1982-
587 56762010000500001

588 Nobile, C.J., Mitchell, A.P., 2007. Microbial biofilms: e pluribus unum. Curr. Biol. 17,
589 349–353. doi:10.1016/j.cub.2007.02.035

590 Ortega-Toro, R., Jiménez, A., Talens, P., Chiralt, A., 2014. Effect of the incorporation
591 of surfactants on the physical properties of corn starch films. Food Hydrocoll. 38, 66–
592 75. doi:10.1016/j.foodhyd.2013.11.011

593 Pastor, C., Sánchez-González, L., Marcilla, A., Chiralt, A., Cháfer, M., González-
594 Martínez, C., 2011. Quality and safety of table grapes coated with
595 hydroxypropylmethylcellulose edible coatings containing propolis extract. Postharvest
596 Biol. Technol. 60, 64–70. doi:10.1016/j.postharvbio.2010.11.003

597 Perdones, A., Sánchez-González, L., Chiralt, A., Vargas, M., 2012. Effect of chitosan-
598 lemon essential oil coatings on storage-keeping quality of strawberry. Postharvest Biol.
599 Technol. 70, 32–41. doi:10.1016/j.postharvbio.2012.04.002

600 Potjewijd, R., Nisperos, M.O., Burns, J.K., Parish, M., Baldwin, E.A., 1995. Cellulose-
601 based coatings as carriers for *Candida guillermondii* and *Debaryomyces sp*. in reducing
602 decay of oranges. HortScience 30, 1417–1421.

603 Reglinski, T., Elmer, P. a G., Taylor, J.T., Wood, P.N., Hoyte, S.M., 2010. Inhibition of
604 *Botrytis cinerea* growth and suppression of botrytis bunch rot in grapes using chitosan.
605 Plant Pathol. 59, 882–890. doi:10.1111/j.1365-3059.2010.02312.x

606 Řiháková, Z., Plocková, M., Filip, V., Šmidrkal, J., 2001. Antifungal activity of lauric
607 acid derivatives against *Aspergillus niger*. Eur. Food Res. Technol. 213, 488–490.
608 doi:10.1007/s002170100416

609 Rodríguez, M., Osés, J., Ziani, K., Maté, J.I., 2006. Combined effect of plasticizers and
610 surfactants on the physical properties of starch based edible films. Food Res. Int. 39,
611 840–846. doi:10.1016/j.foodres.2006.04.002

612 Romanazzi, G., Karabulut, O.A., Smilanick, J.L., 2007. Combination of chitosan and
613 ethanol to control postharvest gray mold of table grapes. Postharvest Biol. Technol. 45,
614 134–140. doi:10.1016/j.postharvbio.2007.01.004

615 Romanazzi, G., Gabler, F.M., Margosan, D., Mackey, B.E., Smilanick, J.L., 2009.
616 Effect of chitosan dissolved in different acids on its ability to control postharvest gray
617 mold of table grape. Phytopathology 99, 1028–1036. doi:10.1094/PHYTO-99-9-1028

618 Sánchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., Cháfer, M.,
619 2009. Characterization of edible films based on hydroxypropylmethylcellulose and tea
620 tree essential oil. Food Hydrocoll. 23, 2102–2109. doi:10.1016/j.foodhyd.2009.05.006

621 Sánchez-González, L., Pastor, C., Vargas, M., Chiralt, A., González-Martínez, C.,
622 Cháfer, M., 2011. Effect of hydroxypropylmethylcellulose and chitosan coatings with
623 and without bergamot essential oil on quality and safety of cold-stored grapes.
624 Postharvest Biol. Technol. 60, 57–63. doi:10.1016/j.postharvbio.2010.11.004

625 Sánchez-González, L., Quintero Saavedra, J.I., Chiralt, A., 2013. Physical properties
626 and antilisterial activity of bioactive edible films containing *Lactobacillus plantarum*.
627 Food Hydrocoll. 33, 92–98. doi:10.1016/j.foodhyd.2013.02.011

628 Teixidó, N., Viñas, I., Usall, J., Magan, N., 1998. Control of blue mold of apples by
629 preharvest application of *Candida sake* grown in media with different water activity.
630 Phytopathology 88, 960–964. doi:10.1094/PHYTO.1998.88.9.960

631 Teixidó, N., Torres, R., Viñas, I., Abadias, M., Usall, J., 2011. Biological control of
632 postharvest diseases in fruit and vegetables, Protective In: Lacroix, C. (ed). Protective
633 cultures, antimicrobial metabolites and bacteriophages for food and beverage
634 biopreservation. Woodhead Publishing Limited, 364-402
635 doi:10.1533/9780857090522.3.364

636 Vargas, M., Pastor, C., Chiralt, A., McClements, D.J., González-Martínez, C., 2008.
637 Recent advances in edible coatings for fresh and minimally processed fruits. Crit. Rev.
638 Food Sci. Nutr. 48, 496–511. doi:10.1080/10408390701537344

639 Viñas, I., Usall, J., Teixidó, N., Sanchis, V., 1998. Biological control of major
640 postharvest pathogens on apple with *Candida sake*. Int. J. Food Microbiol. 40, 9–16.
641 doi:10.1016/S0168-1605(98)00009-9

642 Xu, W.T., Huang, K.L., Guo, F., Qu, W., Yang, J.J., Liang, Z.H., Luo, Y.B., 2007.
643 Postharvest grapefruit seed extract and chitosan treatments of table grapes to control
644 *Botrytis cinerea*. Postharvest Biol. Technol. 46, 86–94.
645 doi:10.1016/j.postharvbio.2007.03.019

646 Zahavi, T., Cohen, L., Weiss, B., Schena, L., Daus, a., Kaplunov, T., Zutkhi, J., Ben-
647 Arie, R., Droby, S., 2000. Biological control of *Botrytis*, *Aspergillus* and *Rhizopus* rots
648 on table and wine grapes in Israel. Postharvest Biol. Technol. 20, 115–124.

649

650 **TABLE CAPTIONS**

651 **Table 1**. Treatments based on different edible coatings and *Candida sake* at $5 \cdot 10^7$ CFU
652 mL^{-1} applied on grapes.

653 **FIGURE CAPTIONS**

654 **Figure 1**. Population of *Candida sake* applied with different coating-forming
655 dispersions on grape surface at 24 h and 7 days after application: CS: *C. sake* in water;
656 hydroxypropymethylcellulose (HPMC), corn starch (S), sodium caseinate (NaCas), pea
657 protein (PP), oleic acid (OA), Span 80 (S80) and Tween 85 (T85). Different letters in
658 the bars indicate significant differences determined using LSD test ($p < 0.05$) for each
659 time. * indicate the treatments that significantly improved the population with respect to
660 CS.

661 **Figure 2**. Percentage of reduction of *Botrytis cinerea* incidence on grape berries by
662 applications of *Candida sake* incorporated in different coating-forming dispersions after
663 7 and 12 days of incubation. CS: C. sake in water; hydroxypropymethylcellulose
664 (HPMC), corn starch (S), sodium caseinate (NaCas), pea protein (PP), oleic acid (OA),
665 Span 80 (S80) and Tween 85 (T85). Different letters in the bars indicate significant
666 differences determined using LSD test ($p < 0.05$) for each time. * indicate the
667 treatments that significantly improved the results of CS treatment.

668 **Figure 3**. Percentage of reduction of *Botrytis cinerea* severity on grape berries by
669 applications of *Candida sake* incorporated in different coating-forming dispersions after
670 12 days of incubation. CS: *C. sake* in water; hydroxypropymethylcellulose (HPMC),
671 corn starch (S), sodium caseinate (NaCas), pea protein (PP), oleic acid (OA), Span 80
672 (S80) and Tween 85 (T85). Different letters in the bars indicate significant differences

673 determined using LSD test ($p < 0.05$) between treatments. * indicate the treatments that
674 significantly improved the results of CS treatment.

675 **Figure 4**. SEM images of coated grape surface with *Candida sake* formulations: water
676 (a, b); sodium caseinate (NaCas) (c, d); corn starch (S) (e, f).

677 **Figure 5**. SEM images of coated grape surface with *Candida sake* formulations: corn
678 starch (S) with oleic acid (OA) at 24 h and 7 days (a, b); S with Tween 85 (T85) at 24 h
679 and 7 days (c, d).

680 **Figure 6**. SEM images of coated grape surface *with Candida sake* formulations: sodium
681 caseinate (NaCas) with oleic acid (OA) at 24 h and 7 days (a, b); NaCas with Tween 85
682 (T85) at 24 h and 7 days (c, d).

683 **Figure 7**. Relative population increase of *Candida sake* with respect to the
684 corresponding control as a function of the amount (mg mL^{-1}) of coating-forming solids
685 with respect to the BCA colonies (5×107 CFU mL^{-1}), for corn starch (S) and sodium
686 caseinate (NaCas) coatings applied on grapes surface after 24 h and 7 days of
687 application. (lines: tendencies of Δlog vs. solid concentration). LSD intervals of the
688 controls at 24 h and 7 days.

689 **Figure 8**. Percentage of reduction of *Botrytis cinerea* incidence on grape berries by
690 applications of *Candida sake* as a function of the amount (mg mL^{-1}) of coating-forming
691 solids with respect to the BCA colonies (5×107 CFU mL^{-1}) after 6 days of incubation.
692 S: corn starch, NaCas: sodium caseinate. Different letters in the bars indicate significant
693 differences determined using LSD test ($p < 0.05$). * indicate the treatments that
694 significantly improved the results of CS treatment.

695 **Figure 9**. Percentage of reduction of *Botrytis cinerea* severity on grape berries by
696 applications of *Candida sake* as a function of the amount (mg mL^{-1}) of coating-forming
697 solids with respect to the BCA colonies (5×107 CFU mL^{-1}) after 10 days of incubation.
698 S: corn starch, NaCas: sodium caseinate. Different letters in the bars indicate significant
699 differences determined using LSD test ($p < 0.05$). * indicate the treatments that
700 significantly improved the results of CS treatment.