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# ENCAPSULATION OF RESVERATROL ON LECITHIN AND β-GLUCANS TO ENHANCE ITS ACTION AGAINST *BOTRYTIS CINEREA*

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**ABSTRACT**

β-glucans and soy lecithin were used as encapsulating materials for resveratrol and tebuconazole, in order to test their antifungal activity against *Botrytis cinerea*. First an oil-in-water emulsion was formed and afterwards the emulsion was dried by spray-drying or by particles from gas saturated solutions-drying (PGSS-drying). β-glucans were precipitated also by supercritical anti-solvent (SAS), but it was not a suitable drying process for this material. Particles were characterized regarding particle size, morphology, crystallinity, encapsulation efficiency and *in vitro* activity against *Botrytis cinerea*. Although the emulsions with β-glucans had bigger droplet size than the ones with lecithin, there was no difference on particle size for each encapsulating material, it was just dependent on the drying process. For all the materials and drying processes, completely amorphous particles were obtained, in spite of the crystalline form of the pure active compounds. Concerning the antifungal activity, the encapsulation of tebuconazole did not improve its action, because it was already very effective. Pristine resveratrol did not reduce fungal growth, but it was inhibited between 50-70% with encapsulated resveratrol, which implies the production of an effective fungicide against *Botrytis cinerea* totally from natural origin substances.

**Keywords:** resveratrol, β-glucan, lecithin, spray-drying, PGSS-drying, fungicide.

## 1. INTRODUCTION

*Botrytis cinerea* is a pathogenic fungus causing gray mold, which affects several fruits and plants all over the world. Once a product is attacked by *Botrytis cinerea*, it cannot be recovered, so it produces great economic losses (Williamson et al., 2007). Also, one of the most important problems when handling with *Botrytis cinerea* is that it infects the plants during the blooming, but it is not noticeable until the ripening (Timperio et al., 2012). Commonly, chemicals are used to fight against this disease, for instance azoles (Stehmann and de Waard, 1996), anilinopyrimidines, phenylpyrroles or hydroxyanilides (Rosslenbroich and Stuebler, 2000). However, resistant strains are developed quickly by the fungus (Elad et al., 1995; Pappas, 1997). Furthermore, as the treatment against *Botrytis cinerea* must be applied in full-grown products, or even post-harvest, there is a tendency towards replacing the use of toxic substances by natural, environmentally friendly products (Ali et al., 2015).

When a plant is attacked by a pathogen, it develops a chemical response which involves the production of some proteins, phytoalexins and other phenolic compounds (Langcake and Pryce, 1976; Montero et al., 2003; Timperio et al., 2012) that show antifungal activity (Mendoza et al., 2013). The main phytoalexin produced is resveratrol, which is found in high quantities on grape skin (Casas et al., 2010; Zheng et al., 2011). Further, it has been found to be active against different fungi (Aldred et al., 2008; Santos et al., 2006), including *Botrytis cinerea* (Adrian et al., 1997). It was observed that, when resveratrol is produced spontaneously to fight against fungi, it is present within the cell wall of the plant to be in contact with the pathogen (Adrian and Jeandet, 2012), or it is even excreted out from the cell in liquid culture (Donnez et al., 2011) to improve the contact between resveratrol and the fungus. Based on this findings, resveratrol has been

selected as promising antifungal of natural origin. Nevertheless, when applied externally in the plant to fight *Botrytis cinerea*, resveratrol should be encapsulated in a carrier that reaches easily the cell wall of the fungi and improves its penetration.

Encapsulation of active substances into polymeric matrices provides a controlled release of the compound, since the encapsulating material can act as a carrier for the active compound into the target, as well as protection against thermal or oxidative degradation. Recently, some works focused on the treatment of *Botrytis cinerea* with chitosan (Badawy and Rabea, 2009; Wu et al., 2005; Xu et al., 2007), which is one of the components of the cell wall of the fungus. Furthermore, the encapsulation of essential oil into chitosan improved its antifungal action against *Botrytis cinerea* (Mohammadi et al., 2015). Taking into account that also β-glucans are present on the cell wall of *Botrytis cinerea* (Tenberge, 2007), this polysaccharide is going to be use as carrier in the present work. β-glucans are composed by D-glucose monomers linked by glycosidic bonds. Depending on the bonds they have, they present different structure and branching, which provides them their properties, such as solubility or biological activity (Bae et al., 2013). To the authors' knowledge, there is just one previous study using β-glucans against *Botrytis cinerea* (Aziz et al., 2003). In this work, linear β-1,3-glucans from seaweeds were used as antifungal compound, and they reduced the growth of the fungus by 50% in 4 days with a concentration of 1 g/L. However, β-glucans have never been used as encapsulating material, although some studies have demonstrated that, when they are added to an emulsion, they increase its stability (Burkus and Temelli, 2000; Kontogiorgos et al., 2004).

77 Encapsulation processes based on supercritical fluids techniques have attracted great
78 interest in the last years, because they allow better control of particle size and
79 morphology and reduce the contamination of the product by working in an inert
80 atmosphere or by an improved removal of the organic solvents (Martín and Cocero,
81 2008). Among the micronization processes with supercritical fluids, particles from gas
82 saturated solutions-drying (PGSS-drying) and supercritical anti-solvent (SAS) are
83 performed in this work.
84 PGSS-drying is a supercritical fluid process suitable for the production of particles, in
85 which the suspension is saturated with $CO_2$ at high pressure and temperature prior to
86 the atomization. The sudden decompression of the fluid in the nozzle promotes the
87 desorption of the $CO_2$ from the droplets, breaking them and enhancing the drying
88 process, so it can be performed at lower temperature than with conventional spray-
89 drying (SD), which prevents the product from thermal degradation (Rodríguez-Rojo et
90 al., 2013). Also, all the process is a closed system inerted with $CO_2$, so it is appropriate
91 for drying substances that are oxidized easily (Martín and Weidner, 2010). Few works
92 used this technology for the production of particles. They were mainly focused on the
93 encapsulation of antioxidants and essential oils (de Paz et al., 2012; Meterc et al., 2008;
94 Varona et al., 2013).
95 Alternatively, in SAS, the encapsulating material and the active compound are dissolved
96 in an organic solvent, and the liquid is introduced through a nozzle in a high pressure
97 vessel containing $CO_2$ in supercritical conditions. At these conditions, $CO_2$ is highly
98 soluble on organic solvents. When the droplets of the liquid stream are put in contact
99 with the supercritical $CO_2$, the organic solvent is saturated with the $CO_2$, so the solubility
100 of the solute decreases and this promotes its precipitation (Rodríguez-Rojo et al., 2013).

101 The aim of this work is to develop the formulation of a product against *Botrytis cinerea*
102 based entirely on natural substances, namely resveratrol, β-glucans and soy lecithin. Soy
103 lecithin has been used as comparison for the β-glucans because it is has been
104 demonstrated that encapsulation essential oils in lecithin can be improved their
105 bactericidal action since it can form liposomes in aqueous media and interact with cells
106 (Varona et al., 2013). Besides, it is biocompatible and non-toxic, and thus it is commonly
107 used in formulation of emulsions in applications related with food processing (de Paz et
108 al., 2012). First, an oil-in-water emulsion of resveratrol on β-glucan, lecithin or a mixture
109 of both substances is produced and afterwards it is dried by conventional SD or by PGSS-
110 drying. Further, tebuconazole, a triazole commonly used in plant protection (Yang et al.,
111 2014), is used to compare the action of resveratrol. Besides, β-glucan particles are also
112 obtained by precipitation by SAS. The particles formed by all these methods are
113 characterized and tested *in vitro* against *Botrytis cinerea*.

114

115 ## 2. MATERIALS AND METHODS

116 ### *2.1. Materials*

117 As encapsulating materials, barley (1-3, 1-4)-β-glucans (75% purity; Glucagel, kindly
118 supplied by DKSH, France) and soybean lecithin (Glama-sot, SOTYA S.A., Madrid, Spain)
119 were used. A molecular weight of 125 kDa was determined by size exclusion
120 chromatography at the conditions indicated on Section 2.3.
121 Ethyl acetate (99%) and malt extract agar were purchased from Panreac (Spain), and
122 dimethylsulfoxide (DMSO) from Sigma-Aldrich (Spain). Resveratrol with 98% purity was
123 purchased from Pure Bulk (USA). Tebuconazole was kindly supplied by Aragonesas Agro

(Spain), both as pure powder (technical grade) and the commercial product Orius 20EW (an oil-in-water emulsion with 20% w/v of tebuconazole).

### *2.2. Formation of particles*

First, an oil-in-water emulsion was formed (IKA Labor Pilot), with the fungicide (tebuconazole or resveratrol) dissolved in ethyl acetate (7.5 g/L) as organic phase, whereas the aqueous phase, saturated with ethyl acetate, contained the different matrixes used (15 g/L): β-glucans, soybean lecithin or a mixture of both of them (50% in weight). Both liquid phases were mixed in a ratio 1:9 at 750 rpm for 5 minutes and then the resulted emulsion was fed into a rotor-stator machine (IKA® LABOR PILOT 2000/4), with 200 mL capacity, and processed at 4200 rpm for 4 minutes. These operating conditions were selected based on some previous works (Varona et al., 2009), where it was found a reduction in droplet size with increasing surfactant concentration, homogenization velocity and time. Afterwards, the organic solvent was removed by vacuum evaporation (Heidolph) at 60º C, 75 rpm and a vacuum of 0.08 MPa, and finally the suspensions were dried either by SD or by PGSS-drying.

By SD (Gea Niro Mobile Minor), the suspension (1 L/h) was introduced into the drying chamber through a rotary atomizer (compressed air at 0.6 MPa). Droplets were formed and water was removed from them by a stream of hot air (130º C at the inlet and 85º C at the outlet). The dry particles were recovered in a cyclone.

By PGSS-drying the suspension was pumped and put into contact with 10 kg/h of preheated and pressurized $CO_2$ (Milton Roy membrane pump) at 9.5 MPa and 125º C in a 150 mm static mixer filled with 4 mm glass beads, so that the liquid was saturated with $CO_2$. Gas to liquid ratio was set at 30 (weight basis). Then this stream was expanded through a nozzle (Spraying Systems, 500 µm) in the drying chamber, which was kept at

65-70º C. The conditions selected for the drying process were based on a previous work (Varona et al., 2011), which contains further details about the experimental device. Figure 1 shows a simplified diagram of the formation of particles by SD and PGSS-drying, with the previous creation of the emulsion and suspension.

FIGURE 1

For the production of β-glucan particles by SAS, DMSO containing β-glucans in a concentration of 2-10 g/L was pumped (HPLC pump, Gilson, model 850) and introduced in a vessel with $CO_2$. Preliminary tests at 20º C showed limited solubility of β-glucans in ethyl acetate, acetone, ethanol and tetrahydrofuran (lower than 2.6 g/L for every solvent), and higher in DMSO (14.8 g/L), so it was the solvent selected. Prior to introduce the solution, the vessel must be conditioned just with $CO_2$ until the required pressure and temperature are achieved. Pressure was always set at 10-11 MPa to avoid the formation of a liquid phase in the precipitation vessel. After all the suspension was pumped, $CO_2$ was still passed through the vessel in order to make sure that there was no remaining DMSO inside. Then, the system was decompressed and the particles formed were recovered from a filter placed at the outlet of the vessel. A scheme of the process is shown in Figure 2.

FIGURE 2

### *2.3. Molecular weight of β-glucans*

Molecular weight of the β-glucans was measured by size exclusion chromatography, with a guard column (Waters Ultrahydrogel Guard Column), a column (Ultrahydrogel 500, Waters Corporation) and a differential refractive index detector (410, Waters

Corporation). The column was kept at 35° C and flow rate of the mobile phase (0.1 M $NaNO_3$ and 0.02% $NaN_3$) was set at 0.4 mL/min.

### *2.4. Morphology of the particles*

The morphology of the particles was analyzed by scanning electron microscopy (SEM, JEOL JSM-820, 20 kV, 23-mm working distance) at vacuum conditions. Prior to the analysis, the samples were covered with gold in an argon atmosphere.

### *2.5. Particle size distribution*

Particle size distribution of the particles and emulsions produced was measured by laser diffraction (Mastersizer 2000, Malvern). In order to perform the measurement, the emulsions were suspended on a solution of water saturated with ethyl acetate, whereas the suspensions were suspended only in water. On the contrary, the dried particles were measured within a stream of air at 0.2 MPa.

### *2.6. DSC*

Differential scanning calorimetry (DSC) profiles of the particles produced by SAS were obtained using a Mettler Toledo 822e calorimeter. Heating rate was 10° C/min in the temperature range of -40 to 280° C. An empty pan was used as reference. The equipment was calibrated with indium.

### *2.7. X-ray diffraction*

X-ray diffraction (XRD) measurements were performed on a Bruker Discover D8 diffractometer to check the crystallinity of the obtained particles, using the Cu Kα radiation (λ = 0.15406 nm). The scattering intensities were measured over an angular range of $5 < 2\theta < 70$ for all the samples, with a step size of 0.02°.

### *2.8. Encapsulation efficiency*

Quantification of tebuconazole and resveratrol in the final particles was done by HPLC, with a guard column (Bio-Sil C18, Bio-Rad), a column (Symmetry C18, Waters) and a UV detector (at 224 nm for tebuconazole and 306 nm for resveratrol). The column was kept at 40º C or 25º C for tebuconazole and resveratrol, respectively, and flow rate of the mobile phase was set at 1 mL/min for tebuconazole (acetonitrile and water) and 0.8 mL/min for resveratrol (acetonitrile and water with formic acid). For each sample, 3 independent measurements were made.

### *2.9. Botrytis cinerea culture*

For the in-vitro culture of *Botrytis cinerea*, the fungus was isolated from vines in our university (Campus La Yutera, Universidad de Valladolid, Palencia, Spain) and it was grown on malt extract agar by two different procedures. In the first one, the fungus was placed on the surface of the solid agar, and the fungicide was dissolved in water with 4% v/v ethanol (96%, Panreac, Spain) and applied to the fungi. The second method consisted on pouring the agar in the dishes containing the particles, so once the agar solidified, the particles were entrapped on it. In all cases, 5 replicates were grown for every sample and the growth area was calculated by measuring the diameter in two directions after incubation at 22º C for one week. Once measured, the average value and standard deviation were evaluated, and Student's t-test was performed (unpaired samples, unequal variances) in order to check whether there were significant differences between each sample, with a significance p-value of 0.05.

## 3. RESULTS AND DISCUSION

### *3.1. Formation of particles*

Prior to the drying process, droplet size distribution of the emulsions and suspensions formed was analyzed (Figure 3).

FIGURE 3

All of the emulsions (Figure 3 (a)) showed unimodal distribution, although a smaller second peak towards bigger particle size is noticed in the case of the particles of lecithin. Lecithin droplets were the smallest ones (d(0.5) = 7.4 ± 0.9 µm). On the contrary, the emulsions created with β-glucans had the biggest droplets (d(0.5) = 52 ± 8 µm). The emulsions formed with a mixture of both substances had an intermediate value of droplet size between those (d(0.5) = 15 ± 5 µm). The formation of bigger droplets with β-glucans is explained by the observation of other authors (Burkus and Temelli, 2000; Kontogiorgos et al., 2004) that they form a network structure when used as stabilizers of emulsions. Similarly, after removing the organic solvent, the same trend in particle size is observed for the suspension: smaller sizes for lecithin and bigger ones for β-glucans formulations (Figure 3 (b)), although particle size is reduced and particle size distribution is broader in all cases.

Then, dry particles were obtained from the suspensions both by SD and by PGSS-drying. All of the samples had moisture content below 6% (water evaporation estimated by weight difference after 24 hours at 105º C; results not shown here). Figure 4 contains SEM images for β-glucan particles obtained by SD (a) and by PGSS-drying (b).

FIGURE 4

244 It is observed that the particles obtained by PGSS-drying had more spherical shape,
245 whereas the ones produced by SD are irregular due to a shrinkage effect caused by the
246 evaporation of water once the crust has started to being formed. This has been also
247 observed by other authors when drying at high temperature (de Barros Fernandes et al.,
248 2014; Porras-Saavedra et al., 2015; Shi and Zhong, 2015), also oat powder suspensions
249 containing between 18-33% (w/w) β-glucans at similar drying conditions (80-82$^{o}$ C in the
250 drying chamber) as the ones used in this work (Chronakis et al., 2004). Neither the
251 particles obtained by SD, nor the ones by PGSS-drying, show cracks in their surface. The
252 morphology results of the PGSS-drying particles are in accordance with the reported
253 ones from previous works (de Paz et al., 2012; Varona et al., 2013). In addition, the
254 particles produced by PGSS-drying are smaller than by SD, but they form agglomerates
255 very easily, producing much larger clusters. This is in accordance with the results
256 reported by other authors (Li et al., 2013), who detected an increase of the
257 agglomeration of particles when drying at lower temperature. The formation of small
258 particles by PGSS-drying is a consequence of the desorption of $CO_2$ from the liquid when
259 the pressure is suddenly reduced in the nozzle, which breaks the liquid into smaller
260 droplets. Figure 5 shows particle size distributions of the particles formed with the
261 different encapsulating materials by both SD and PGSS-drying.

262 FIGURE 5

263

264 According to these results, despite the different droplet size of the emulsions as a
265 function of the encapsulating material, particle size was just dependent on the drying
266 process: it was in the range of 10 μm for SD and 150 μm for PGSS-drying. This confirms

SEM results that, even though smaller primary particles were created by PGSS-drying, they formed huge agglomerates.

Regarding the formation of particles by SAS, the operating conditions of the different experiments performed are shown In Table 1:

TABLE 1

Except for experiments B, C and D, which were performed at higher temperature, the rest of them were done at 10 MPa and 35º C in order to have one single phase for the mixture of DMSO and $CO_2$, according to the data reported by other authors (Andreatta et al., 2007).

By SAS it was not possible to obtain well dried β-glucan particles in the operating conditions tested. At higher temperature, no solid material was obtained. In the experiments at lower temperature, the product recovered from the vessel formed a matrix rather than particles. This is observed with SEM images (figure 4 c and d): there are melted micro-particles (4c) which conform aggregates with particle size around 1 mm (4d).

By DSC it was estimated that after the drying process there was still between 20-30% (w/w) of DMSO in the final product (DSC results not shown), so it was not completely extracted with supercritical $CO_2$. Several previous studies succeeded on obtaining solid particles by SAS using DMSO with different biopolymers, as dextrans, poly-(hydroxypropylmethacrylamide) (HPMA) (Reverchon et al., 2000), cyclodextrins (De Marco and Reverchon, 2008) or hemicellulosic material (Haimer et al., 2010). However, in all cases, molecular weight of the biopolymers tested was lower than that of the β-glucans used in this work. Thus, with β-glucans, DMSO was entrapped into the chains of

polymer, the viscosity of the solution increased and it was not possible to extract it with $CO_2$ in the selected operating conditions. This problem when precipitating high viscous solutions of DMSO in supercritical fluids was also observed by other authors (Fernández-Ronco et al., 2014).

### *3.2. Loading of active compounds in the particles*

Results of the loading of tebuconazole and resveratrol for the different encapsulating materials and drying processes are shown on Table 2.

TABLE 2

The recovery of the active compound is higher for tebuconazole by SD rather than by PGSS-drying. On the contrary, when using resveratrol PGSS-drying is more effective, because in PGSS-drying lower drying temperature is required and the product is protected from oxidation since it is included in an inert atmosphere of $CO_2$. Anyway, the differences between both processes are lower than 10%, except in the case of the particles of resveratrol encapsulated on β-glucans (recovery 30% higher by PGSS-drying). These small differences are probably due to the fact that the encapsulation of the active compound into the carrier is done by emulsification prior to the drying process, so there is not influence of the latter on the loading of tebuconazole and resveratrol in the final particles.

When comparing the different encapsulating materials, the loading of active compound is always higher for lecithin than for β-glucans, again with the exception of the case mentioned above. However, the values are high (above 49%) with both materials, probably due to the formation of hydrogen bonds between the active compounds and the encapsulating materials, as pointed out in some previous works for the case of

lecithin with antioxidants (Gonçalves, 2015) and polyphenols with β-glucans (Wu et al., 2011).

Furthermore, XRD analysis were performed in order to check whether there were crystals of fungicide remaining on the surface of the particles or they were completely encapsulated. In all cases, the peaks corresponding to tebuconazole or resveratrol were observed in the physical mixtures with the encapsulating material, but they could not be noticed in the particles obtained either by SD or by PGSS-drying. As an example, figure 6 shows the XRD diagram for the particles of resveratrol and lecithin obtained by SD and the physical mixture of both compounds with the same composition.

FIGURE 6

The particles obtained do not show any of the peaks corresponding to crystals of resveratrol. As the structure is totally amorphous, it is assume that resveratrol is correctly included into the lecithin and there is not unbound crystals of resveratrol in the sample. In addition, the encapsulated resveratrol is on amorphous state too, since XRD is a penetrating technique and the corresponding diffraction peaks were not observed. This same behavior is observed for all the particles produced, and it is in accordance with previous results of β-glucan complexes with some nutraceuticals (Veverka et al., 2014).

### *3.3. Antifungal activity against Botrytis cinerea*

According to commercial guidelines of Tebuconazole agricultural products, it is recommended to be applied at a concentration between 100 and 250 mg/L, so the antifungal tests were performed at 100 mg/L of tebuconazole. Figure 7 (a) shows the results of the growth of the fungus when applying pure tebuconazole (Teb), a

commercial oil-in-water emulsion (Orius), and the particles of lecithin (Lec), a mixture of lecithin and β-glucans (LecbG) and β-glucans (bG) produced by SD (SD) or PGSS-drying (PGSS).

FIGURE 7

All the particles obtained produced a great inhibition of the fungal growth (between 75 and 80% compared to the control sample). However, no significant differences were observed with the commercial tebuconazole products. Therefore, although the encapsulated particles were very effective against *Botrytis cinerea* growth, they did not improve the action of powder and oil-in-water commercial tebuconazole, because it is already very active against this fungus. Figure 7 shows some images of the Petri dishes of *Botrytis* culture as control (b), with commercial tebuconazole powder (c) and with the particles of tebuconazole encapsulated on β-glucan obtained by SD (d) and by PGSS-drying (e). It is noticed that growth is reduced when applying both pure tebuconazole and the particles, compared to the control dishes, but there are not significant differences among them.

For the activity of resveratrol, it was considered that previous works (Adrian et al., 1997) reported growth inhibition of *Botrytis cinerea* with resveratrol concentration between 60 and 160 mg/L, so 100 mg/L of resveratrol was chosen for the in vitro tests. Figure 8 (a) reveals the results corresponding to the growth of *Botrytis cinerea* in the presence of pure resveratrol (Resv), pure soy lecithin (Lec), pure β-glucans (bG) and a mixture of both substances (LecbG), and the particles created with these encapsulating materials and pure resveratrol by SD (SD) or PGSS-drying (PGSS).

362 FIGURE 8

363

364 No effect was observed on fungal growth when applying pure resveratrol, although
365 growth inhibition at that concentration range was reported in previous works (Adrian et
366 al., 1997). The main difference is that here it was applied as pure crystals, whereas in
367 that work it was dissolved in an ethanolic solution. However, the growth was reduced
368 between 50 and 70% by all the particles of resveratrol produced in this work. This means
369 that the encapsulation of resveratrol enhanced its antifungal activity. Small differences
370 were noticed between both drying processes for every encapsulating material, although
371 particles of lecithin were more effective by SD, while particles of β-glucans resulted
372 slightly better by PGSS-drying.

373 Figure 8 contains images of the growth in Petri dishes of some samples. Similar growth
374 is observed between the control sample (b), the one with pure resveratrol powder (c)
375 and the one with pure β-glucan (d). However, the growth area is smaller for the particles
376 of resveratrol encapsulated on β-glucans by SD (e), and even smaller for the ones by
377 PGSS-drying (f).

378 Pure β-glucans did not affect the growth of the fungus, but lecithin reduced it by 40%
379 (figure 8 (a)). Still, the mixture of both compounds had also no effect on growth
380 inhibition. Taking this into account, the effectiveness of the particles encapsulated on
381 lecithin relays greatly on lecithin. Nevertheless, the action of the particles encapsulated
382 on β-glucans and a mixture of lecithin and β-glucans is related to the synergistic effect
383 of these substances and resveratrol, because neither of them showed effect when
384 applied alone, as well as to the change of resveratrol from crystals to amorphous state

after processing, determined by XRD. Therefore, the shell material improved the absorption of resveratrol, which in this way was active against the *Botrytis cinerea*.

## 4. CONCLUSIONS

Resveratrol and tebuconazole were formulated as emulsions with lecithin, β-glucans and mixtures thereof. Formulations were successfully dried by SD and PGSS-drying. However, SAS was not a suitable process to obtain β-glucan particles in the conditions tested, because the product had quantities of remaining organic solvent up to 30% (w/w). Final particle size by SD was in the range of 10 µm and in PGSS-drying in the range of 100 µm, showing that the drying processes and not the carrier material determined the final particle size. As first there was an emulsification step, the loading of active compound in the particles was very similar for both drying processes. Furthermore, the interaction of the active compounds with the carriers produced their precipitation in the encapsulated particles in amorphous state instead of their usual crystalline form. This change in crystallinity improved the antifungal activity of resveratrol, which showed no effect against *Botrytis cinerea* as pure crystals, but reduced the fungal growth between 50 and 70% with all resveratrol particles. However, pure and commercial emulsion of tebuconazole inhibited fungal growth by 75-80%, so no further effect could be observed with the particles of encapsulated tebuconazole.

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**FIGURE CAPTIONS**

Figure 1. Formation of particles by SD and PGSS-drying.

Figure 2. SAS process.

Figure 3. Droplet size distribution of the emulsions (a) and suspensions (b) of resveratrol with different encapsulating material.

Figure 4. SEM images of β-glucan particles by SD (a), PGSS-drying (b) and by SAS (exp. F: 5 g/L in DMSO, precipitation at 10 MPa, 35º C, 2 mL/min; c and d).

Figure 5. Droplet size distribution of the particles produced by SD and PGSS-drying with different encapsulating material.

Figure 6. Physical mixture and SD particles of resveratrol and lecithin.

Figure 7. Growth area of *Botrytis cinerea* for the products of tebuconazole (a) and images of the growth area in the Petri dishes: control (b), commercial tebuconazole (c), tebuconazole and β-glucan particles by SD (d) and by PGSS-drying (e).

Figure 8. Growth area of *Botrytis cinerea* for the products of resveratrol (a) and images of the growth area in the Petri dishes: control (b), pure resveratrol (c), β-glucans (d), resveratrol and β-glucan particles by SD (e) and by PGSS-drying (f).

**FIGURES**

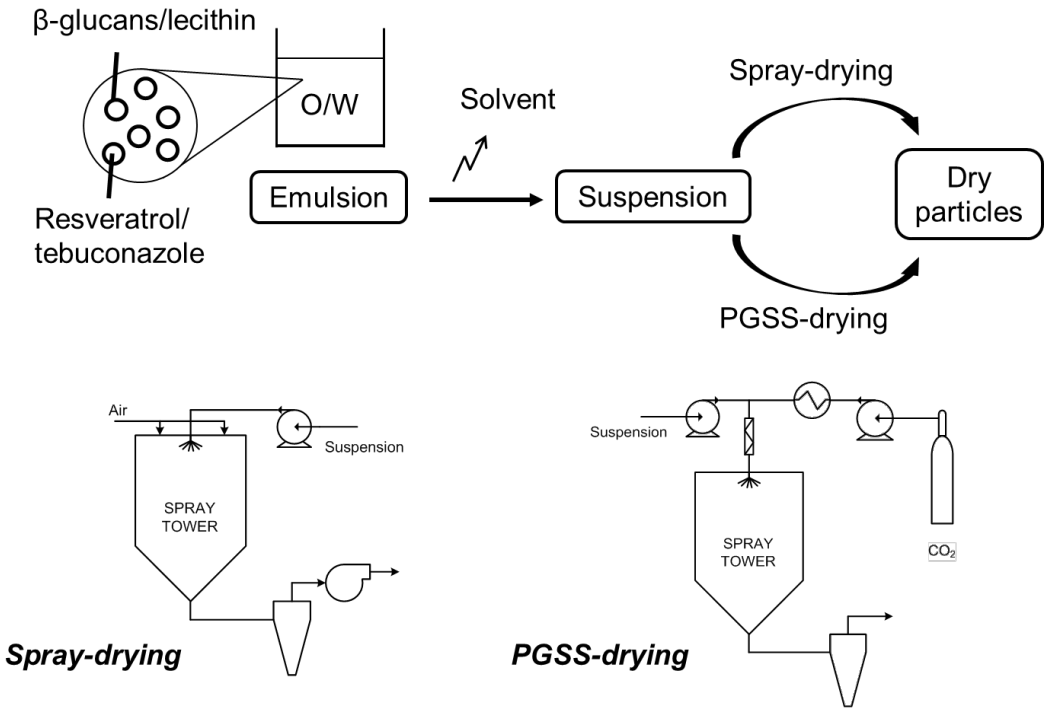


***Figure 1.*** *Formation of particles by SD and PGSS-drying.*

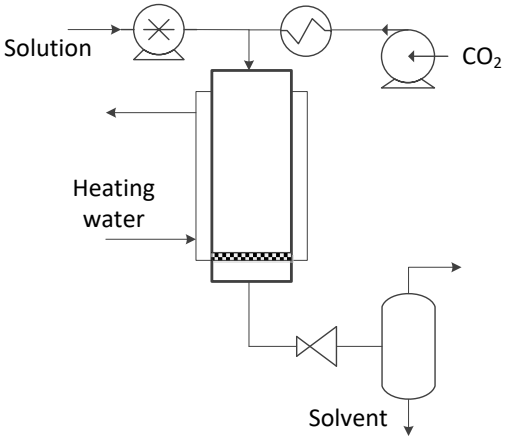


***Figure 2.*** *SAS process.*

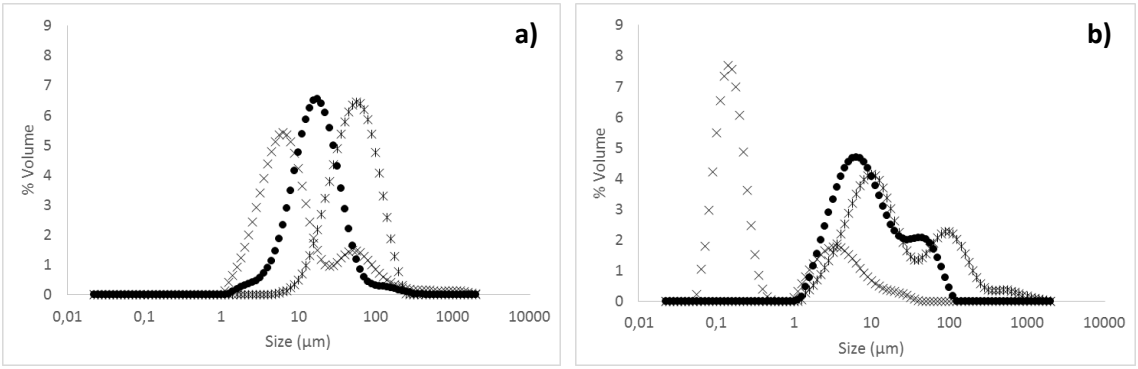


***Figure 3.*** *Droplet size distribution of the emulsions (a) and suspensions (b) of resveratrol with different encapsulating material. X: lecithin; ⚹: β-glucan; ●: lecithin and β-glucan.*

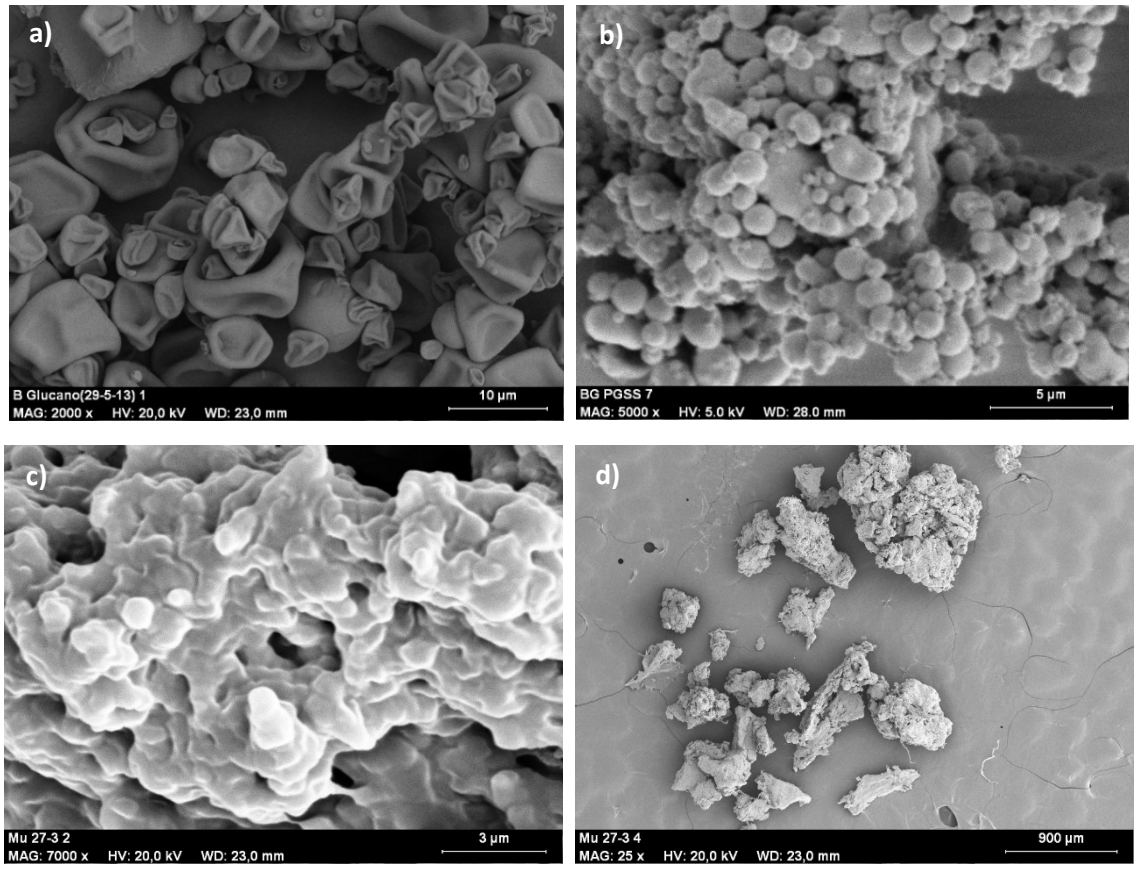


***Figure 4.*** *SEM images of β-glucan particles by SD (a), PGSS-drying (b) and by SAS (exp. F: 5 g/L in DMSO, precipitation at 10 MPa, 35° C, 2 mL/min; c and d).*

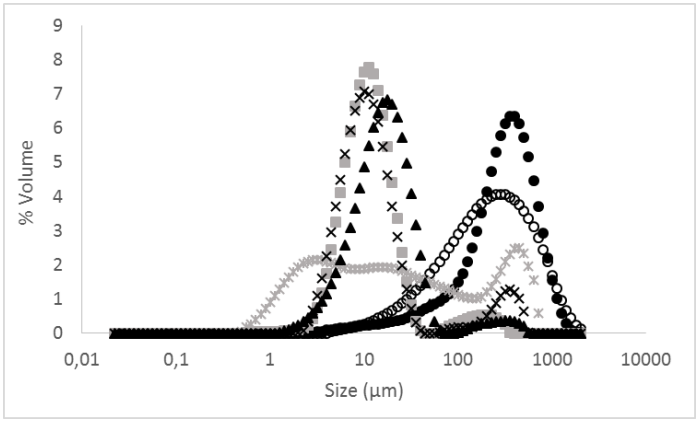


***Figure 5.*** *Droplet size distribution of the particles produced by SD and PGSS-drying with different encapsulating material. By PGSS-drying: ○ : with lecithin; ● : with lecithin and β-glucans; X: with β-glucans; and by SD ■ :with lecithin; ▲: with lecithin and β-glucans; and X: with β-glucans.*

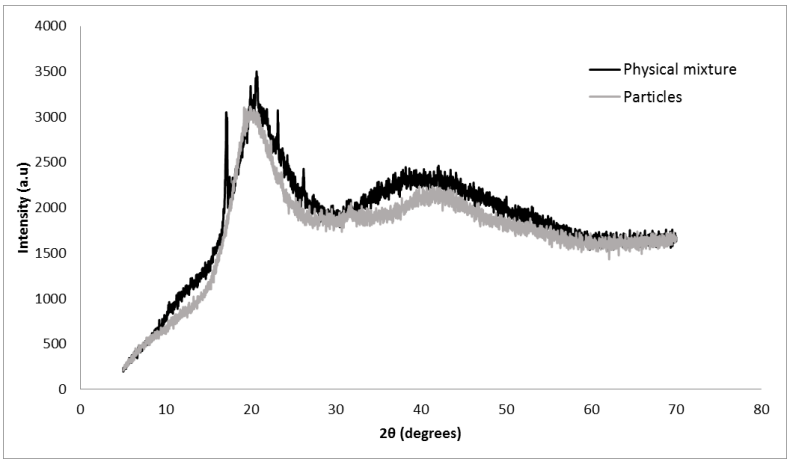


***Figure 6.*** *Physical mixture and SD particles of resveratrol and lecithin.*

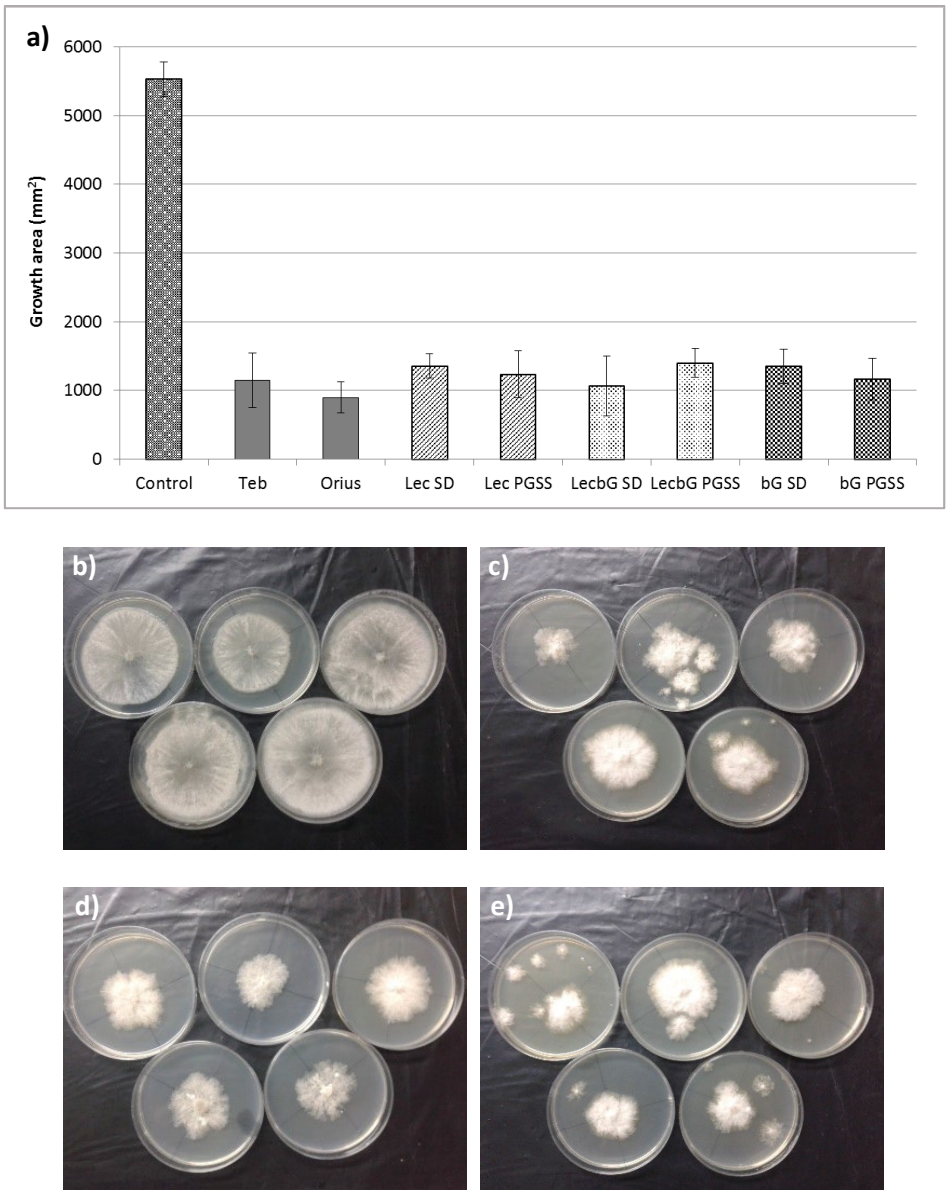


***Figure 7.*** *Growth area of Botrytis cinerea for the products of tebuconazole (a) and images of the growth area in the Petri dishes: control (b), commercial tebuconazole (c), tebuconazole and β-glucan particles by SD (d) and by PGSS-drying (e).*

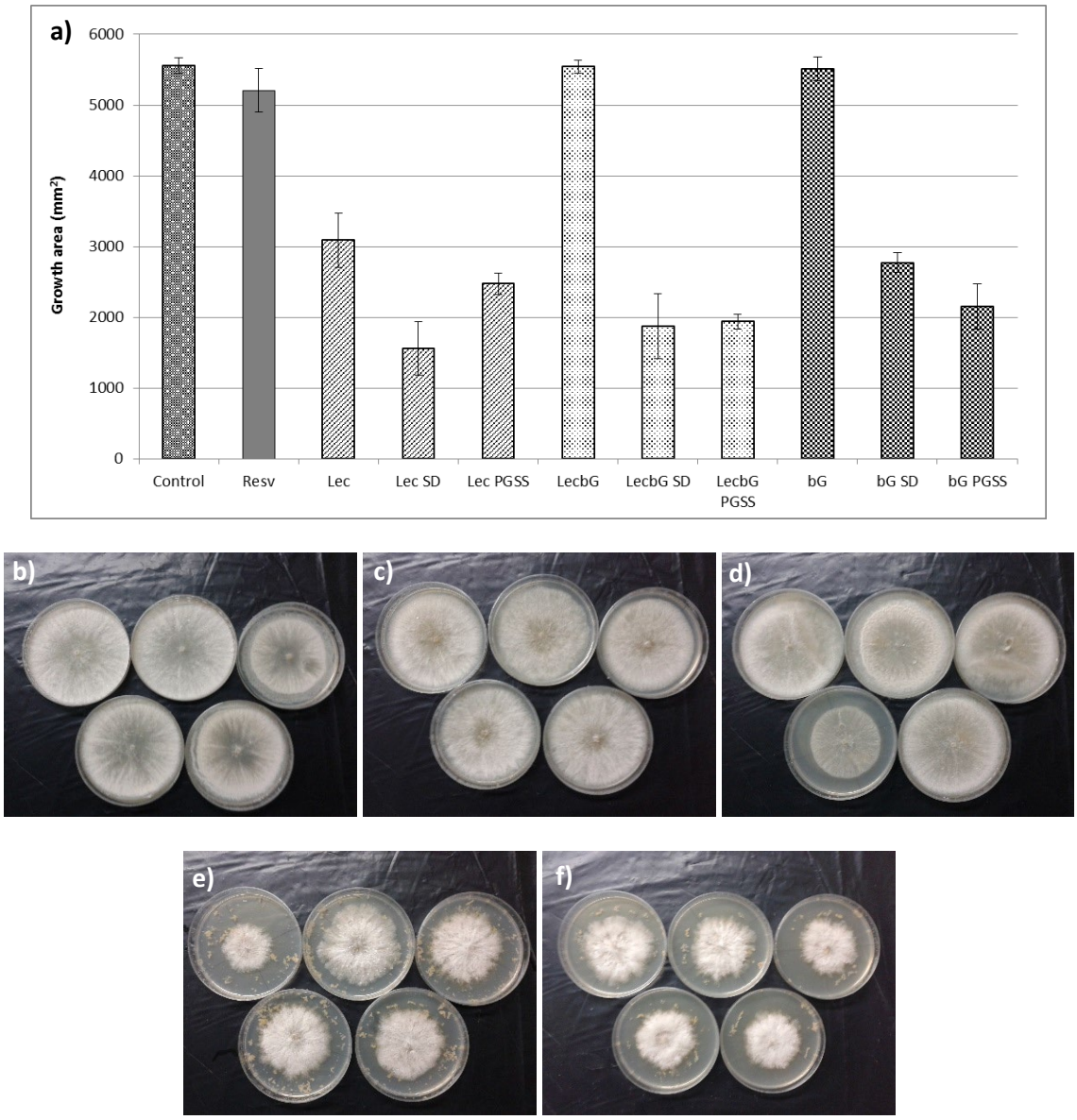


***Figure 8.*** *Growth area of Botrytis cinerea for the products of resveratrol (a) and images of the growth area in the Petri dishes: control (b), pure resveratrol (c), β-glucans (d), resveratrol and β-glucan particles by SD (e) and by PGSS-drying (f).*

**TABLE CAPTIONS**

Table 1. SAS experiments.

Table 2. Fungicide loading for the different materials and drying processes.

**TABLES**

***Table 1.*** *SAS experiments.*

| Exp | β-glucan concentration ($g/L_{DMSO}$) | Dissolution flow (mL/min) | $CO_2$ flow (kg/h) | T (ºC) |
|---|---|---|---|---|
| A | 2 | 2 | 2.5 | 34-35 |
| B | 5 | 2 | 2.5 | 37 |
| C | 5 | 2 | 2.5 | 38 |
| D | 5 | 2 | 2.5 | 50 |
| E | 5 | 2 | 2.5 | 34-35 |
| F | 5 | 2 | 5 | 34-35 |
| G | 5 | 4 | 2.5 | 34-35 |
| H | 10 | 2 | 2.5 | 34-35 |

***Table 2.*** *Fungicide loading for the different materials and drying processes.*

| % loading | Tebuconazole | | Resveratrol | |
|---|---|---|---|---|
| | SD | PGSS-drying | SD | PGSS-drying |
| Lecithin | 86.4 ± 1.1 | 79.1 ± 1.9 | 71 ± 4 | 75.3 ± 0.2 |
| Lecithin and β-glucans | 82.6 ± 1.6 | 72 ± 9 | 65 ± 7 | 67 ± 2 |
| β-glucans | 57 ± 2 | 49 ± 14 | 61.3 ± 1.2 | 94 ± 2 |