ENCAPSULATION OF FUNGICIDE TO ENHANCE ITS PENETRATION THROUGH THE CELL WALL OF THE FUNGI

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ABSTRACT

Particles of fungicide encapsulated in lecithin and β-glucans were obtained in order to improve the action of the product against *Botrytis cinerea*. For this purpose, supercritical drying processes such as PGSS and SAS were performed and compared to conventional spray-drying. It was concluded that SAS is not a suitable process for these products. However, particles were well dried by any of the other two methods. High recovery of tebuconazole was achieved, especially for the particles that contained lecithin. The formulation was active against *Botrytis*, though there were not significant differences between all the formulations tested.

INTRODUCTION

Botrytis cinerea is a pathogenic fungus that affects several fruits and plants all over the world. Commonly, chemicals are used to fight against this disease [1]. However, in order to avoid the formation of strains resistant to fungicides and to reduce the use of toxic substances in an effort to preserve the environment, new formulation methods are being developed. One of the alternatives is the encapsulation of fungicides and pesticides, since it is assumed that in this way the effect of the product is higher than that of the conventional product or it is released slower.

In this work, β-glucans are used as encapsulating material for conventional fungicides. Taking into account that the cell wall of *Botrytis cinerea* contains β-glucans in its structure [2], this novel formulation may provide an improvement on the absorption of the fungicide in the fungus, which results in more effectiveness and less quantity of product required to control the infection.

Triazole is a family of compounds commonly employed in plant protection, because it is useful for the treatment of several fungi. However, if too much product is used, it can be phytotoxic and retard plant growth [3]. In this work, tebuconazole is used because, among triazoles, it is the most widely used in agriculture.

The drying processes used in this work to obtain particles are conventional spray-drying and particles from gas saturated solutions (PGSS-drying). In PGSS-drying the suspension to be

dried is first saturated with supercritical CO_2 and then atomized. Particles are formed due to two effects of the sudden decompression: the desorption of the CO_2 from the droplets and the evaporation of water. Besides, because of the Joule-Thomson effect, temperature decreases after the expansion, so particles can be obtained at mild temperature, preventing the product from thermal degradation [4]. This is the main advantage of PGSS-drying in comparison to spray-drying.

Besides, particles are obtained also by supercritical anti-solvent technique (SAS). In this process, when the droplets of an organic solvent containing a solute are put in contact with supercritical CO_2, the organic solvent is saturated with the CO_2, so the solubility of the solute decreases and this promotes its precipitation [4].

Once the particles are produced by any of the above mentioned methods, the encapsulation efficiency of tebuconazole in the different matrixes and drying processes is evaluated. The morphology of the particles is analyzed by scanning electron microscopy (SEM). Also the antifungal activity of the particles against *Botrytis cinerea* is studied through in-vitro culture techniques, and compared to that of pure tebuconazole powder and commercial emulsion of tebuconazole.

MATERIALS AND METHODS

Materials

As encapsulating materials, barley β-glucans with a purity of 75% and molecular weight of 125 kDa (Glucagel TM, DKSH, France) and soybean lecithin (Glama-sot, SOTYA S.A.) were used. Tebuconazole was kindly supplied by Aragonesas Agro S.A., both pure (technical grade) and as an oil-in-water emulsion with 20% w/v of tebuconazole (ORIUS 20EW). Ethyl acetate with a purity of 99% (Panreac) and dimethylsulfoxide (DMSO, Sigma-Aldrich) were used as organic solvents.

Formation of particles

First, an oil-in-water emulsion was formed (IKA Labor Pilot), with tebuconazole dissolved in ethyl acetate (7,5 g/L) as organic phase, whereas the aqueous phase contained the different matrixes used (15 g/L): β-glucans, soybean lecithin or a mixture of both of them. Then, the organic solvent was removed by vacuum evaporation (Heidolph) and finally the suspensions were dried.

In spray-drying (Gea Niro Mobile Minor), the suspension (1 L/h) was introduced into the drying chamber through a rotary atomizer (compressed air at 6 bar). Droplets were formed and water was removed from them by a stream of hot air (130º C at the inlet and around 83º C at the outlet). The dry particles were recovered in a cyclone.

In PGSS-drying, the suspension was pumped and put into contact with preheated and pressurized CO_2 (Milton Roy membrane pump) at 95 bar and 125º C in a 150 mm static mixer filled with 4 mm glass beads, so that the liquid was saturated with CO_2. Then this stream was expanded through a nozzle (0,5 mm) in the drying chamber, which was kept at 65-70º C.

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 at 100 bar and 35º C. After all the suspension was pumped, the system was decompressed and the particles formed were recovered from a filter placed at the outlet of the vessel.

Morphology of the particles

The morphology of the particles was analized by SEM (JEOL JSM-820).

Encapsulation efficiency

Quantification of tebuconazole 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). The column was kept at 40º C and flow rate of the mobile phase (acetonitrile and water) was set at 1 mL/min.

Botrytis cinerea culture

For the in-vitro culture of *Botrytis cinerea*, the fungus was obtained from vines in our university (Campus La Yutera) and it was grown on malt extract agar (Cultimed, Panreac). The fungus was placed on it, and the fungicide was dissolved in water with 4% v/v ethanol (96%, Panreac) and applied to the fungi. The growth area was calculated by measuring the diameter after incubation at 22ºC for one week.

RESULTS

Dry particles were obtained both by spray-drying and by PGSS, but not by SAS. It can be easily observed with the images of SEM (figure 1): β-glucans dried on this way still contained organic solvent and were highly agglomerated. This might be due to the high viscosity of the solution of DMSO. On the contrary, the particles obtained by any of the other methods were better dried and, though they may agglomerate, they have smaller size (between 1-10 μm).

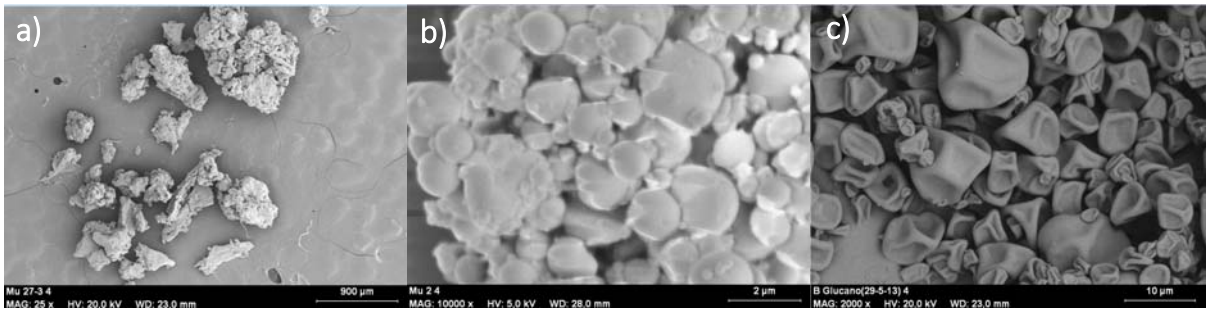

Figure 1: β-glucan particles obtained by SAS (a), PGSS-drying (b) and spray-drying (c)

The analysis of the tebuconazole content reveals similar results in the particles dried by spray-drying and PGSS-drying for each encapsulating material, although it is slightly lower in the particles from PGSS-drying. The results are shown in the following table, being % encapsulation the quantity of tebuconazole in the particles related to the initial quantity in the emulsion:

Table 1: Percentage of the initial tebuconazole in the final dry particles

% encapsulation		
	Spray-drying	PGSS-drying
Lecithin	86,4	77,7
Lecithin and β-glucans	82,6	78,1
β-glucans	56,7	53,7

For both processes, the lowest efficiency is obtained for the β-glucan particles, while the quantity obtained for the particles with lecithin is almost the same as for the particles with both β-glucans and lecithin.

Concerning the antifungal action of the particles, all of them reduced the growth of *Botrytis cinerea*, which was just 20-25% of the growth without any treatment. However, there was no improvement on the effect of commercial tebuconazole. This is observed in the following figure, where the results for all the particles obtained both by spray-drying (SD) and PGSS-drying (PGSS) and the commercial fungicide in a concentration of 0,1 g/L of tebuconazole are represented.

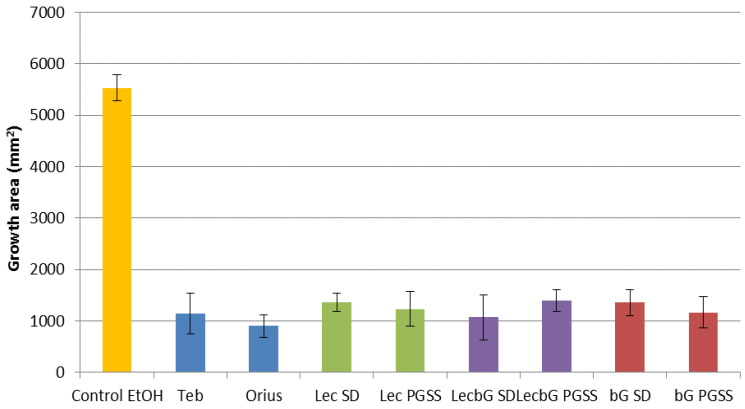

Figure 2: *Botrytis* growth after one week.

CONCLUSION

Tebuconazole particles formulated with lecithin and β-glucans were produced by spray-drying and PGSS-drying. The particles obtained were similar independently of the drying method: they had good shape and high content of tebuconazole. They were also effective against *Botrytis cinerea*, though the encapsulation did not improve the action of commercial tebuconazole.

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