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Evaluation of the microencapsulation process of conidia of *Trichoderma asperellum* by spray drying

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Abstract

Microencapsulation of biological control agents by spray drying (SD) has been studied as a method for increasing product shelf life and stability to enable the application of microencapsulated agents in sustainable agriculture. In this study, the microencapsulation of *Trichoderma asperellum* conidia by spray drying was evaluated. The objective was to assess the influence of drying air temperature and wall material (maltodextrin DE20, MD20) concentration on the microencapsulation of *Trichoderma asperellum* conidia and to identify the optimum conditions to produce. Microparticles were characterized in terms of morphology, particle size, and shelf life. A central composite rotatable design (CCRD) was used to investigate the effect of operating parameters on drying yield (DY), moisture content, conidial viability (CV), and percentage of conidial survival (SP). Microencapsulation experiments were carried out under optimum conditions to validate the obtained model. The optimum temperature and MD20/conidia dry weight ratio were 80°C and 1:4.5, respectively, which afforded a drying yield of 63.85 ± 0.86%, a moisture content of 4.92 ± 0.07%, a conidial viability of 87.10 ± 1.16%, and a conidial survival of 85.78 ± 2.88%. Microencapsulation by spray drying using MD20 as wall material extended the viability of conidia stored at 29°C compared with the control.

Key points:

- *Trichoderma asperellum* conidia were microencapsulated by spray drying;
- Temperatures higher than 100°C negatively affected *T. asperellum*;
- Scanning electron microscopies showed that maltodextrin well covered the conidia.

Keywords: Microparticle; powder; wall material; central rotational compound design; biological control.

Introduction

Sustainable agronomy and environmental protection are important issues nowadays (Schmoll and Schuster 2010; Wang et al. 2017; Bacior and Prus 2018; Li et al. 2018). Fungi of the genus *Trichoderma* spp. show potential for plant growth promotion (Youssef et al. 2016; Jalali et al. 2017; Zhang et al. 2017) and are the main species used for biological control of phytopathogens. *Trichoderma* can interact with phytopathogens through different mechanisms, such as parasitism, antibiosis, and competition, and have a resistance-inducing effect on plants against diseases (Chen et al. 2016; Szczech et al. 2017; Vinodkumar et al. 2017).

Compared with chemical agents, *Trichoderma* products have some disadvantages, particularly with respect to shelf life and viability during storage and field application (Jin and Custis 2011; Muñoz-Celaya et al. 2012; Locatelli et al. 2018). High water activity, high drying temperatures during processing (Jin and Custis 2011), prolonged storage, high storage temperatures (Muñoz-Celaya et al. 2012; Broeckx et al. 2017; Reyes et al. 2018), contamination of growth medium, and oxidative stress (Reyes et al. 2018) are factors that affect the viability of fungal formulations.

Microencapsulation of microorganisms by spray drying (SD) is a valuable technique for producing microbial formulations in powder form. During spray drying, the product temperature is kept low by the rapid evaporation of water from droplets, which makes the technique suitable for drying heat-sensitive products (Gharsallaoui et al. 2007) without compromising quality. Spray drying has been shown to increase the shelf life of fungal preparations (Jin and Custis 2011; Muñoz-Celaya et al. 2012; Ma et al. 2015; Broeckx et al. 2017; Reyes et al. 2018; Braga et al. 2019). However, inappropriate selection of operational parameters, such as inlet air temperature, wall material concentration, liquid feed flow rate, and drying air flow rate can result in low conidial survival, unsuitable particle size and morphology as well as high moisture content and water activity (Sosnika and Seremet, 2015; Kemp et al. 2016; Cotabarren et al. 2018).

Because of the large number of variables that can influence a spray-drying process, studies use statistical design and mathematical optimization models to describe drying and

microencapsulation processes of microorganisms and determine how much variables contribute to product quality and their interaction effects (Chandramouli et al. 2004; Chávez and Ledeboer 2007; Koc et al. 2010; Anekella and Orsat 2013; Behboudi-Jobbehdar et al. 2013; Da Costa et al. 2015; Braga et al. 2019). The use of statistical experimental design reduces the number of experiments required to study a given process, thereby reducing the time and financial resources needed to conduct an experimental investigation (Box et al. 2005).

The objectives of this work were to investigate the influence of drying air temperature and maltodextrin concentration on the characteristics of *Trichoderma asperellum* conidial powders produced by spray drying; to determine optimum process conditions for conidial viability; to characterize the obtained material by physicochemical, microbiological, and morphological tests; and to evaluate the storage stability of *T. asperellum* conidial powders produced with and without wall material by spray drying under optimized conditions.

Material and methods

T. asperellum conidial suspensions and wall material

T. asperellum conidial suspensions, produced by solid-state fermentation, and wall material, maltodextrin DE20 (Galena, Brazil), were kindly provided by Farroupilha Lallemand Biocontrol Laboratory (Patos de Minas, Brazil). Conidial suspensions were characterized for moisture content, conidial concentration, germination, and colony forming unit (CFU) count prior to each experiment.

Microencapsulation by spray drying

The *T. asperellum* conidia and maltodextrin mixture was maintained at room temperature under constant stirring while being fed to the spray dryer (MSD 1.0, LabMaq, Brazil). Experiments were conducted using a feed rate of 0.6 L h^{-1}, drying air flow rate of 9.90 $\times 10^4$ L h^{-1}, and atomization air flow rate of 2,400 L h^{-1}.

To optimize process conditions, we used a central composite rotatable design (CCRD), with three replicates at the center point and 11 treatments in total. Inlet air temperature and maltodextrin concentration were the independent variables. Drying yield, microparticle moisture content, conidial viability, and conidial survival were the dependent variables.

100 Variables and their levels are presented in Table 1. The upper and lower limits of
101 independent variables were determined according to preliminary tests and previous studies on
102 microencapsulation of *Trichoderma* spp. Conidia (Jin and Custis 2011; Muñoz-Celaya et al.
103 2012).
104
105 Insert Table 1
106
107 To perform the microbiological analyzes, the powders were rehydrated up to the same
108 spore concentration of the initial solution fed to the dryer and characterized for germination and
109 colony forming units (CFU), with the main objective of evaluating the amount of viable conidia
110 (CV) and percentage of conidia (SP) survival.
111
112 *Process optimization and experimental validation*
113 Optimum operational conditions for microencapsulation of *T. asperellum* conidia by
114 spray drying were determined by response surface methodology (RSM) based on CCRD, as
115 described by Box et al. (2005). Model equations were imported into MATLAB, contour plots
116 were constructed and superimposed, and the region of optimum response for all variables was
117 identified.

118 *Physicochemical, microbiological and morphological analyzes*
119
120 Microbiological analyses were performed using powders rehydrated to the initial spore
121 concentration of the feed solution. Germination percentage was determined to assess conidial
122 viability, and CFU counts were used as a measure of conidial survival.
123 *T. asperellum* conidial suspensions were characterized for spore concentration,
124 germination percentage, and CFU count. Microparticles were characterized for moisture
125 content (A.O.A.C. (Association of Official Analytical Chemists) 2005), spore concentration,
126 germination percentage, and CFU count.
127 Conidia were counted using a Neubauer chamber. Conidial viability after spray drying
128 was determined using the germination test proposed by Danielson and Davey (1973) and
129 Milner et al. (1991) , with modifications. CFU counting was carried out according to the method
130 of Jin and Custis (2011). Viable conidia concentration was expressed as CFU per gram.
131 Conidial survival percentage was calculated as the CFU count of the feed solution divided by
132 the CFU count of spray-dried powders times 100 (Picot and Lacroix 2004).

Microscopic examination of powder samples was performed using a sputter coater (Leica, Germany) and a conventional scanning electron microscope (Zeiss, Germany). Particle size distribution was evaluated by laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK).

Drying yield was calculated as the ratio of the dry weight of powder samples to the dry weight of feed solutions (*T. asperellum* conidial suspension with or without wall material).

T. asperellum conidial viability before and during storage

Conidial viability was determined by CFU counting before and during storage using microencapsulated conidia (test sample) and a control sample (without wall material). Samples were placed in packaging of the aluminum, stored at 29°C in a BOD incubator (Ethik Technology, Brazil), and evaluated on days 7, 34, 68, 73, 90, 122, and 129 of storage. Prior to viability analysis, samples were homogenized by vortexing, and a 1 g aliquot was mixed with 9 mL of sterile water, according to Reyes et al. (2018).

Statistical analysis

Student's *t*-test was applied to compare differences between CCRD results (drying yield, moisture content, conidial viability, and conidial survival) at a significance level of 0.10. Data were analyzed using Statistica software.

Results

Central composite rotatable design (CCRD)

The results of drying yield (%), moisture (%), viable conidia (%) and percentage of survival (%) as a function of the experimental conditions of the CCRD using maltodextrin DE20 as the wall material are shown in Table 2.

Insert Table 2

Insert Figure 1

The response surface plots of drying yield as a function of inlet air temperature and maltodextrin concentration are presented in the Fig. 1(a). The Eq. 1 and Fig. 1(a) show that an increase in inlet air temperature had a positive effect on drying yield. Accordingly, treatment 5, which was conducted at the lowest temperature (51.8°C), resulted in the lowest yield (36.50%).

The Eq. 1 describes the drying yield as a function of the significant ($p < 0.10$) variable:

$$DY\ (\%) = 61.00 + 9.68T_i \quad (1)$$

where DY is the drying yield and T_i is the inlet air temperature.

Treatments 1 and 5, performed using the lowest inlet air temperatures (60 and 51.8°C, respectively) and wall material/conidial dry weight ratios, resulted in powders with high moisture content (about 7%). In treatment 2, a wall material/conidial dry weight ratio of 1:8.4 was used, and the lowest moisture content (5.87%) was obtained. The model equation (Eq. 2) for moisture content in microparticles is given by:

$$Moisture\ (\%) = 3.78 - 1.75T_i \quad (2)$$

where T_i is the inlet air temperature.

The response surface plot (Fig. 1b) shows that the increase in inlet air temperature caused a significant decrease in microparticle moisture content. The effect of maltodextrin concentration on microparticle moisture was not significant ($p > 0.10$). The coefficient of determination (R^2) for the response variable moisture content was 0.88; that is, the model explained 88% of the variance in moisture content. Samples with 8% moisture were obtained at 50–60°C, whereas, at higher temperatures (100 to 110°C), samples with 2–3% moisture were obtained. However, the use of inlet air temperatures above 100 °C is not recommended because it results in a low percentage of conidial survival. Decrease in conidial viability occurred mainly because of the increase in temperature, as shown by Eq. (3).

$$CV\ (\%) = 83.68 - 35.30T_i - 23.08T_i^2 \quad (3)$$

where CV is the conidial viability percentage, T_i is the inlet air temperature, and T_i^2 is the maltodextrin/conidia dry weight ratio.

The response surface of conidial viability (Fig. 1c) ($R^2 = 0.93$) shows that the highest responses were obtained at inlet air temperatures of 55 to 75°C and wall material/conidia dry weight ratios of 1:3 to 1:10.

Eq. (4) describes conidial survival as a function of inlet air temperature and maltodextrin concentration:

$$CS\ (\%) = 81.81 - 32.19T_i - 22.24T_i^2 \quad (4)$$

where CS is the conidial survival percentage, T_i is the inlet air temperature, and T_i^2 is the maltodextrin/conidia dry weight ratio.

According to Eq. (4), an increase in inlet air temperature causes a decrease in conidial survival. The coefficient of determination (R^2) for the response variable was 0.95; that is, the model explained 95% of the variance in conidial survival. Jin and Custis (2011) showed that

194 the best conidial survival results were obtained using inlet air temperatures of 50–80°C, whereas
195 the worst results were obtained using temperatures of 120–140°C.
196 The response surface plot of conidial survival (Fig.1d) revealed that inlet air
197 temperatures of 60 to 70°C and wall material/conidia dry weight ratios of 1:5 to 1:9 gave the
198 highest conidial survival percentages.
199

200 *Optimization of process conditions and validation of the model*
201

202 Contour plots were overlaid using MATLAB to determine the optimum operating
203 conditions (Fig. 2) for obtaining microparticles with low moisture content, high conidial
204 viability, and high conidial survival. Conidial viability and survival values were evaluated
205 considering moisture percentages less than 6% and drying yields higher than 50%.
206

207 Insert Figure
208

209 The Fig. 2 shows that optimum responses were achieved with inlet air temperatures of
210 77 to 79°C and wall material/conidia dry weight ratios of 1:3.8 to 1:7. An inlet air temperature
211 of 80°C was chosen as the optimum temperature, as it was within the operating range of the
212 spray dryer used. The optimum wall material/conidia dry weight ratio was defined as 1:4.5.
213 New tests were carried out using these parameters to confirm the results and validate the model.
214 Eqs. (1–4) were used to calculate the predicted response values. The goodness of fit of the
215 models was evaluated by the coefficient of determination (R^2) and residue analysis. Table 3
216 shows the predicted and experimental data used to validate the models.
217 Insert Table 3
218

219 Under optimized conditions, drying yield of 63.85 ± 0.86%, moisture content of 4.92
220 ± 0.07%, conidial viability of 87.10 ± 1.16%, and conidial survival of 85.78 ± 2.88% were
221 obtained. These results were satisfactory in comparison with those of the literature.
222 Experimental data were very similar to predicted values. Thus, drying yield, conidial viability,
223 and conidial survival equations were adequate to predict responses at reliable levels. However,
224 we emphasize that these models are valid for the studied experimental range only.
225

226 *Morphology of microencapsulated T. asperellum conidia*
227

The Scanning Electron Microscopy (SEM) images of *T. asperellum* conidia microencapsulated by spray drying using maltodextrin DE20 as wall material under optimum operating conditions is evaluated in Fig. 3.

Insert Figure 3

Particle size distribution

The particle size distribution of *T. asperellum* conidia microparticles obtained using optimum parameters is evaluated in Table 4.

Insert Table 4

In this study, the use of maltodextrin DE20 as wall material increased the mean D50 and D90 values. We also observed that particles smaller than the mean size of pure conidia (D10 = 1.56 μm) were formed, supposedly being particles formed only with maltodextrin. Particle size distribution was similar to that obtained by Jin and Custis (2011), 10–25 μm, and Ma et al. (2015), 7–14 μm, who used the spray-drying technique to obtain microencapsulated *Trichoderma harzianum* and *B. subtilis*, respectively.

Viability of T. asperellum conidia before and during storage

After 7 days of storage at 29°C, reduction in conidia germination was less significant in microencapsulated conidia than in the control, 4.97×10^9 CFU g^{-1} to 3.37×10^9 CFU g^{-1} compared with 2.17×10^{10} CFU g^{-1} to 9.53×10^9 CFU g^{-1}. Similar results were obtained after 34 days of storage, which confirms that the addition of maltodextrin DE20 to the spray-drying process contributed to the preservation of conidia during drying and, consequently, to conidial viability throughout storage at 29°C. The Fig. 4 shows the CFU count of microencapsulated *T. asperellum* conidia and the control sample during the storage period.

Insert Figure 4

Discussion

In the present study of central composite rotatable design (CCRD) yields ranged from 36.5% (treatment 5) to 76.37% (treatment 6) as shown in Table 2. Zhou et al. 2004 investigated the effects of inlet air temperature on the yield of *Bacillus thuringiensis* powder obtained by spray drying. Drying yields of 65.55% and 78.52% were obtained using inlet air temperatures of 180°C and 210°C, respectively, a sample feed rate of 60 mL min^{-1}, and an atomization air pressure of 0.10 MPa.

In the work of LeClair et al. (2016), inlet air temperature and feed solute concentration were significant variables for the spray-drying yield of thermally stable viral vectors. Powder yield varied from 90 to 50%, and the best results were obtained at temperatures close to 120°C. Behboudi-Jobbehdar et al. (2013) studied the spray-drying yield of *Lactobacillus acidophilus* microencapsules by varying inlet air temperature (120, 140, and 160°C) and feed rate (6.0, 7.5, and 9.0 mL min^{-1}). The authors observed that maximum yield (about 70%) was obtained at high drying temperatures and low feed rates. However, it is known that high temperature conditions in spray-drying microencapsulation decrease conidial survival, which is one of the most important parameters to be optimized.

Jin and Custis (2011) reported that the lowest *T. harzianum* conidial survival was obtained at an inlet air temperature of 140°C and the highest, at 60°C. In the same study, the authors investigated the microencapsulation of *T. harzianum* conidia at 40 to 140°C and observed that water condensed on the walls of the drying chamber at low inlet air temperatures, which indicated that the process was not adequate. The same result was observed in the present study at an inlet temperature of 51.8°C (treatment 5) in Table 2. At high temperatures, however, no problems were observed, in accordance with the reported by Jin and Custis (2011). As shown in Table 2, high inlet air temperatures (100.00 and 108.20°C in treatments 3, 4, and 6) negatively influenced conidial viability and survival. A high survival percentage (approximately 80%) was obtained at 80°C and wall material/conidia dry weight ratios above 1:4.9. In a study by Ma et al. (2015), the survival of microencapsulated *B. subtilis* with maltodextrin as wall material was higher than 90% after spray drying at 145°C with a feed rate of 0.55 L h^{-1}. However, it is known that bacteria are less thermosensitive than fungi, which explains the high survival capacity of this microorganism compared with that of *Trichoderma asperellum* found in the present study.

290 Concerning the SEM images of Morphology of microencapsulated conidia showed that
291 a dense and robust structure surrounded *T. asperellum* conidia. Most particles had an irregular
292 matrix structure with a wrinkled appearance and concave depressions. According to Lian et al.
293 (2002) and Favaro-Trindade et al. (2010), concave shapes are characteristic of atomized
294 particles. According to Rodríguez-Huezo et al. (2007), the formation of dimples is due to the
295 drying temperature used. Lian et al. (2002) reported that wall material affects dimple size. The
296 morphology of the microparticles obtained in the present work is similar to that of
297 microcapsules of *T. harzianum* conidia obtained by Muñoz-Celaya et al. (2012) by spray drying
298 using maltodextrin DE10 at 20% (w/v) as wall material.
299 As conidia were well covered by the wall material and as an intermediate inlet air
300 temperature (80°C) was used, good results of conidial viability (87.10 ± 1.16%) and conidial
301 survival (85.78 ± 2.88%) were obtained. Jin and Custis (2011) and Muñoz-Celaya et al. (2012)
302 obtained conidial survival percentages of 76% and 86% using sucrose and maltodextrin as wall
303 material, respectively, for the microencapsulation of *T. harzianum*.
304 The viability of *T. asperellum* conidia before and during storage was evaluated with
305 CFU. The CFU count of microencapsulated conidia (5.33×10^8 CFU g^{-1}) and control ($1.50 \times$
306 10^8 CFU g^{-1}) at 68 days of storage was significantly lower than the initial count, and conidial
307 survival was 10.74 ± 1.16% and 0.69 ± 0.61%, respectively. After 129 days of storage, the
308 number of viable conidia was significantly lower in both samples, 2.70×10^6 CFU g^{-1} for
309 microencapsulated conidia and 8.67×10^6 CFU g^{-1} for the control. According to Harman and
310 Custis (2015), it is ideal that *Trichoderma* formulations contain 5×10^9 CFU g^{-1} to be effective
311 in a variety of applications. According to a study by EFSA (EFSA (European Food Safety
312 Authority) 2013), however, the minimum number of viable *Trichoderma* spp. conidia can vary
313 depending on the type of application and crop.
314 In the work by Muñoz-Celaya et al. (2012), the viability of spray-dried *T. harzianum*
315 conidia without wall material decreased significantly after 4 weeks of storage at 4 and 29°C.
316 However, when using maltodextrin DE10 and gum Arabic as wall materials, conidial survival
317 decreased only after 8 weeks of storage (conidial survival percentages of 40% and 23% at 4°C
318 and 29°C, respectively). Other studies evaluating the survival of spray-dried microorganisms at
319 different storage temperatures showed that microorganisms remained more stable under
320 refrigeration (Paéz et al. 2012; Barbosa et al. 2016; Reyes et al. 2018). Domingues et al. (2016)

evaluated mycelial growth of *T. asperellum* conidia during storage at 12–27°C and found that mycelial growth was directly proportional to the increase in temperature. The authors stated that low temperatures favored the latency of fungi.

Semyonov et al. (2011) evaluated the stability of microcapsules of *Lactobacillus casei* subsp. produced by spray drying during storage at different temperatures, 4, 25, and 37°C. The wall materials were composed of maltodextrins (DE5 and DE19) and a trehalose and maltodextrin mixture. The authors concluded that high storage temperatures affected significantly the survival of microorganisms. After 7 and 28 days of storage at 37 and 25°C, survival was considerably lost, whereas, after 40 days of storage at 4°C, viability was above 70%. Another external factor that influenced probiotic survival was oxygen. Samples stored under nitrogen at 25°C maintained greater viability than samples stored in an air atmosphere. Oxidation of membrane lipids can lead to the production of hydroperoxides and the formation of toxic compounds, damaging microbial DNA (J. Marnett et al. 1985; Akasaka 1986). Chávez and Ledeboer (2007) also reported high cell viability using low oxygen levels during storage of probiotic microorganisms.

Muñoz-Celaya et al. (2012) reported that the increase in shelf life of microencapsulated *T. harzianum* conidia was due to the presence of biopolymers in the formulation, as these materials can delay the diffusion of oxygen for approximately 8 weeks of storage, reducing sample oxidation and oxidative stress.

The optimum operating conditions for microencapsulation of *T. asperellum* conidia by spray drying were determined, which can contribute to improving the industrial feasibility of the process. The optimum inlet air temperature was defined as 80°C and optimum wall material/conidia dry weight ratio was 1:4.5. Mathematical models were able to predict accurately the drying yield, microparticle moisture content, conidial viability, and conidial survival. Under optimum inlet air temperature and maltodextrin concentration conditions, conidial viability and survival were 87.10 ± 1.16% and 85.78 ± 2.88%, respectively. Furthermore, spray-drying microencapsulation using maltodextrin DE20 as wall material was able to extend conidia shelf life compared with the control.

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Contributions

A.B.A.C.B. and C.J.M.C performed physicochemical, microbiological, and morphological analyzes and microencapsulation by spray drying. A.B.A.C.B. realized the process optimization and experimental validation and *Trichoderma asperellum* conidial viability before and during storage and realized the statistical analysis. M.F.Z; E.J.R. and L.D.S. helped in performing the experiments, designed and conceived the studies, and helped in revising the manuscript. All authors contributed to the article and have read and agreed to the published version of the manuscript.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical statement

Conflict of Interest: Author Alinne Brandão Andalécio Camargos Braga declares that she has no conflict of interest. Author Cleiver Junio Martins Costa declares that he has no conflict of interest. Author Eloízio Júlio Ribeiro declares that he has no conflict of interest. Author Marta Fernanda Zotarelli declares that she has no conflict of interest. Author Líbia Diniz Santos declares that she has no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

References

A.O.A.C. (Association of Official Analytical Chemists) (2005) Official methods of analysis of the association of official analytical chemists, 18th edn. Gathersburg

Akasaka S (1986) Inactivation of transforming activity of plasmid DNA by lipid peroxidation. BBA - Gene Struct Expr 867:201–208 . https://doi.org/10.1016/0167-4781(86)90035-7

Alejandro Sosnika, Katia P. Seremet (2015) Advantages and challenges of the spray-drying

technology for the production of pure drug particles and drug-loaded polymeric carriers. Adv Colloid Interface Sci 223:40–54

Anekella K, Orsat V (2013) Optimization of microencapsulation of probiotics in raspberry juice by spray drying. LWT - Food Sci Technol 50:17–24 . https://doi.org/10.1016/j.lwt.2012.08.003

Bacior S, Prus B (2018) Infrastructure development and its influence on agricultural land and regional sustainable development. Ecol Inform 44:82–93 . https://doi.org/10.1016/j.ecoinf.2018.02.001

Barbosa J, Borges S, Teixeira P (2016) Effect of Different Conditions of Growth and Storage on the Cell Counts of Two Lactic Acid Bacteria after Spray Drying in Orange Juice. Beverages 2:8 . https://doi.org/10.3390/beverages2020008

Behboudi-Jobbehdar S, Soukoulis C, Yonekura L, Fisk I (2013) Optimization of Spray-Drying Process Conditions for the Production of Maximally Viable Microencapsulated L. acidophilus NCIMB 701748. Dry Technol 31:1274–1283 . https://doi.org/10.1080/07373937.2013.788509

Box GE, Hunter HG, Hunter JS (2005) Statistics for Experimenters: Design, Innovation, and Discovery. John Wiley & Sons, New York

Braga ABAC, Costa CJM, Pomella AWV, Ribeiro EJ, Santos LD, Zotarelli MF (2019) Evaluation of lethality temperature and use of different wall materials in the microencapsulation process of Trichoderma asperellum conidias by spray drying. Powder Technol 347:199–206 . https://doi.org/10.1016/j.powtec.2019.02.037

Broeckx G, Vandenheuvel D, Henkens T, Kiekens S, van den Broek MFL, Lebeer S, Kiekens F (2017) Enhancing the viability of Lactobacillus rhamnosus GG after spray drying and during storage. Int J Pharm 534:35–41 . https://doi.org/10.1016/j.ijpharm.2017.09.075

Chandramouli V, Kailasapathy K, Peiris P, Jones M (2004) An improved method of microencapsulation and its evaluation to protect Lactobacillus spp. in simulated gastric conditions. J Microbiol Methods 56:27–35 . https://doi.org/10.1016/j.mimet.2003.09.002

Chávez BE, Ledeboer AM (2007) Drying of probiotics: Optimization of formulation and process to enhance storage survival. Dry Technol 25:1193–1201 . https://doi.org/10.1080/07373930701438576

Chen JL, Sun SZ, Miao CP, Wu K, Chen YW, Xu LH, Guan HL, Zhao LX (2016) Endophytic *Trichoderma gamsii* YIM PH30019: A promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on

418 root-rot pathogenic fungi of Panax notoginseng. J Ginseng Res 40:315–324 .
419 https://doi.org/10.1016/j.jgr.2015.09.006
420 Cotabarren IM, Bertín D, Razuc M, Ramírez-Rigo MV, Piña J (2018) Modelling of the spray
421 drying process for particle design. Chem Eng Res Des 132:1091–1104 .
422 https://doi.org/10.1016/j.cherd.2018.01.012
423 Da Costa JMG, Silva EK, Toledo Hijo AAC, Azevedo VM, Malta MR, Ferreira Alves JGL,
424 Borges S V. (2015) Microencapsulation of Swiss cheese bioaroma by spray-drying:
425 Process optimization and characterization of particles. Powder Technol 274:296–304 .
426 https://doi.org/10.1016/j.powtec.2015.01.037
427 Danielson RM, Davey CB (1973) Effects of nutrients and acidity on phialospore germination
428 of Trichoderma in vitro. Soil Biol Biochem 5:517–524 . https://doi.org/10.1016/0038-
429 0717(73)90041-2
430 Domingues MVPF, Moura KE de, Salomão D, Elias LM, Patricio FRA (2016) Effect of
431 temperature on mycelial growth of Trichoderma, Sclerotinia minor and S. sclerotiorum, as
432 well as on mycoparasitism. Summa Phytopathol 42:222–227 .
433 https://doi.org/10.1590/0100-5405/2146
434 EFSA (European Food Safety Authority) (2013) Conclusion on the peer review of the pesticide
435 risk assessment of the active substance *Trichoderma gamsii* ICC080. In: EFSA J.
436 Favaro-Trindade CS, Santana AS, Monterrey-Quintero ES, Trindade MA, Netto FM (2010)
437 The use of spray drying technology to reduce bitter taste of casein hydrolysate. Food
438 Hydrocoll 24:336–340 . https://doi.org/10.1016/j.foodhyd.2009.10.012
439 Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R (2007) Applications of spray-
440 drying in microencapsulation of food ingredients: An overview. Food Res Int 40:1107–
441 1121 . https://doi.org/10.1016/j.foodres.2007.07.004
442 Harman GE, Custis DB (2015) Formulations of viable microorganisms and their methods of
443 production and use
444 J. Marnett L, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN (1985) Naturally
445 occurring carbonyl compounds are mutagens Salmonella tester strain TA104. Mutat Res
446 Mol Mech Mutagen 148:25–34 . https://doi.org/10.1016/0027-5107(85)90204-0
447 Jalali F, Zafari D, Salari H (2017) Volatile organic compounds of some Trichoderma spp.
448 increase growth and induce salt tolerance in Arabidopsis thaliana. Fungal Ecol 29:67–75 .
449 https://doi.org/10.1016/j.funeco.2017.06.007
450 Jin X, Custis D (2011) Microencapsulating aerial conidia of *Trichoderma harzianum* through

spray drying at elevated temperatures. Biol Control 56:202–208 . https://doi.org/10.1016/j.biocontrol.2010.11.008

Kemp IC, Hartwig T, Hamilton P, Wadley R, Bisten A (2016) Production of fine lactose particles from organic solvent in laboratory and commercial-scale spray dryers. Dry Technol 34:830–842 . https://doi.org/10.1080/07373937.2015.1084314

Koc B, Yilmazer MS, Balkir P, Ertekin FK (2010) Spray drying of yogurt: Optimization of process conditions for improving viability and other quality attributes. Dry Technol 28:495–507 . https://doi.org/10.1080/07373931003613809

LeClair DA, Cranston ED, Xing Z, Thompson MR (2016) Optimization of Spray Drying Conditions for Yield, Particle Size and Biological Activity of Thermally Stable Viral Vectors. Pharm Res 33:2763–2776 . https://doi.org/10.1007/s11095-016-2003-4

Li C xi, Ma S chen, Shao Y, Ma S tian, Zhang L ling (2018) Effects of long-term organic fertilization on soil microbiologic characteristics, yield and sustainable production of winter wheat. J Integr Agric 17:210–219 . https://doi.org/10.1016/S2095-3119(17)61740-4

Lian WC, Hsiao HC, Chou CC (2002) Survival of bifidobacteria after spray-drying. Int J Food Microbiol 74:79–86 . https://doi.org/10.1016/S0168-1605(01)00733-4

Locatelli GO, dos Santos GF, Botelho PS, Finkler CLL, Bueno LA (2018) Development of Trichoderma sp. formulations in encapsulated granules (CG) and evaluation of conidia shelf-life. Biol Control 117:21–29 . https://doi.org/10.1016/j.biocontrol.2017.08.020

Ma X, Wang X, Cheng J, Nie X, Yu X, Zhao Y, Wang W (2015) Microencapsulation of Bacillus subtilis B99-2 and its biocontrol efficiency against Rhizoctonia solani in tomato. Biol Control 90:34–41 . https://doi.org/10.1016/j.biocontrol.2015.05.013

Milner RJ, Huppatz RJ, Swaris SC (1991) A new method for assessment of germination of Metarhizium conidia. J Invertebr Pathol 57:121–123 . https://doi.org/10.1016/0022-2011(91)90048-U

Muñoz-Celaya AL, Ortiz-García M, Vernon-Carter EJ, Jauregui-Rincón J, Galindo E, Serrano-Carreón L (2012) Spray-drying microencapsulation of Trichoderma harzianum conidias in carbohydrate polymers matrices. Carbohydr Polym 88:1141–1148 . https://doi.org/10.1016/j.carbpol.2011.12.030

Paéz R, Lavari L, Vinderola G, Audero G, Cuatrin A, Zaritzky N, Reinheimer J (2012) Effect of heat treatment and spray drying on lactobacilli viability and resistance to simulated gastrointestinal digestion. Food Res Int 48:748–754 .

https://doi.org/10.1016/j.foodres.2012.06.018

Picot A, Lacroix C (2004) Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. Int Dairy J 14:505–515 . https://doi.org/10.1016/j.idairyj.2003.10.008

Reyes V, Chotiko A, Chouljenko A, Campbell V, Liu C, Theegala C, Sathivel S (2018) Influence of wall material on production of spray dried lactobacillus plantarum nrrl b-4496 and its viability at different storage conditions. Dry Technol 36:1738–1748 . https://doi.org/10.1080/07373937.2017.1423324

Rodríguez-Huezo ME, Durán-Lugo R, Prado-Barragán LA, Cruz-Sosa F, Lobato-Calleros C, Alvarez-Ramírez J, Vernon-Carter EJ (2007) Pre-selection of protective colloids for enhanced viability of Bifidobacterium bifidum following spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic. Food Res Int 40:1299–1306 . https://doi.org/10.1016/j.foodres.2007.09.001

Schmoll M, Schuster A (2010) Biology and biotechnology of Trichoderma. Appl Microbiol Biotechnol 87:787–799 . https://doi.org/10.1007/s00253-010-2632-1

Semyonov D, Ramon O, Shimoni E (2011) Using ultrasonic vacuum spray dryer to produce highly viable dry probiotics. LWT - Food Sci Technol 44:1844–1852 . https://doi.org/10.1016/j.lwt.2011.03.021

Szczech M, Nawrocka J, Felczyński K, Małolepsza U, Sobolewski J, Kowalska B, Maciorowski R, Jas K, Kancelista A (2017) Trichoderma atroviride TRS25 isolate reduces downy mildew and induces systemic defence responses in cucumber in field conditions. Sci Hortic (Amsterdam) 224:17–26 . https://doi.org/10.1016/j.scienta.2017.05.035

Vinodkumar S, Indumathi T, Nakkeeran S (2017) Trichoderma asperellum (NVTA2) as a potential antagonist for the management of stem rot in carnation under protected cultivation. Biol Control 113:58–64 . https://doi.org/10.1016/j.biocontrol.2017.07.001

Wang X, Liu F lai, Jiang D (2017) Priming: A promising strategy for crop production in response to future climate. J Integr Agric 16:2709–2716 . https://doi.org/10.1016/S2095-3119(17)61786-6

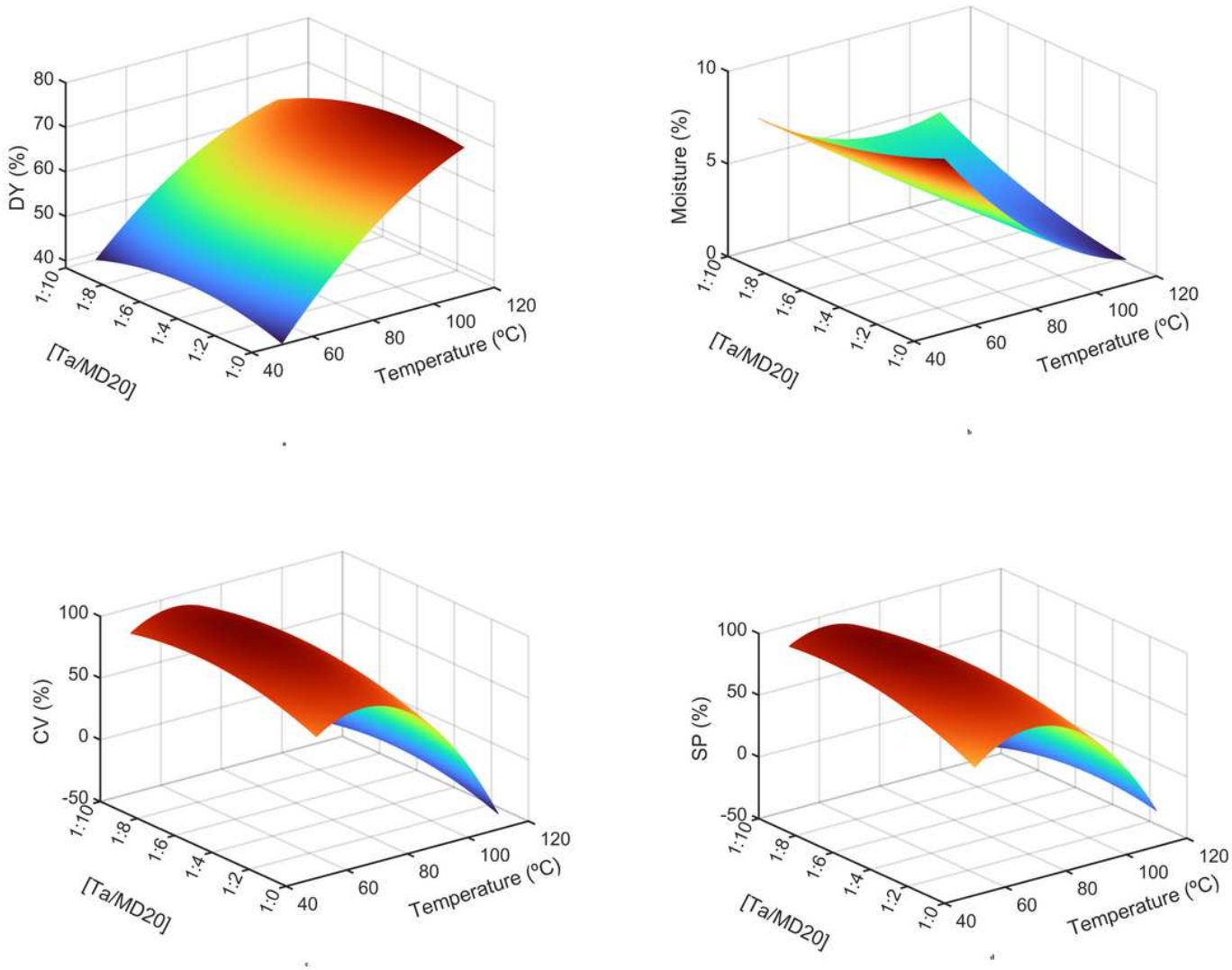
Youssef SA, Tartoura KA, Abdelraouf GA (2016) Evaluation of Trichoderma harzianum and Serratia proteamaculans effect on disease suppression, stimulation of ROS-scavenging enzymes and improving tomato growth infected by Rhizoctonia solani. Biol Control 100:79–86 . https://doi.org/10.1016/j.biocontrol.2016.06.001

Zhang F, Chen C, Zhang F, Gao L, Liu J, Chen L, Fan X, Liu C, Zhang K, He Y, Chen C, Ji X

(2017) *Trichoderma harzianum* containing 1-aminocyclopropane-1-carboxylate deaminase and chitinase improved growth and diminished adverse effect caused by *Fusarium oxysporum* in soybean. J Plant Physiol 210:84–94 . https://doi.org/10.1016/j.jplph.2016.10.012

Zhou X, Chen S, Yu Z (2004) Effects of spray drying parameters on the processing of a fermentation liquor. Biosyst Eng 88:193–199 . https://doi.org/10.1016/j.biosystemseng.2004.02.004

Figures



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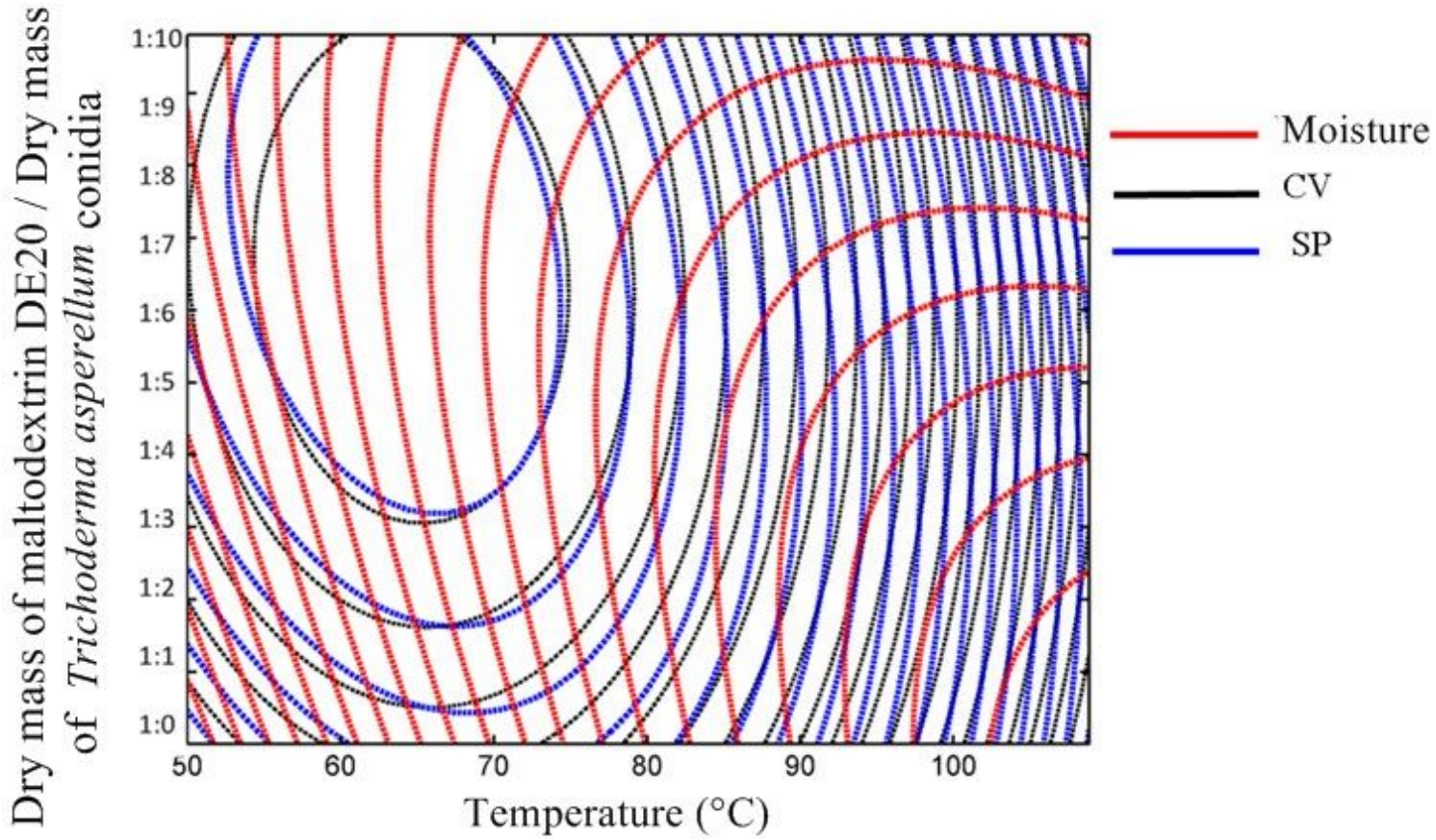

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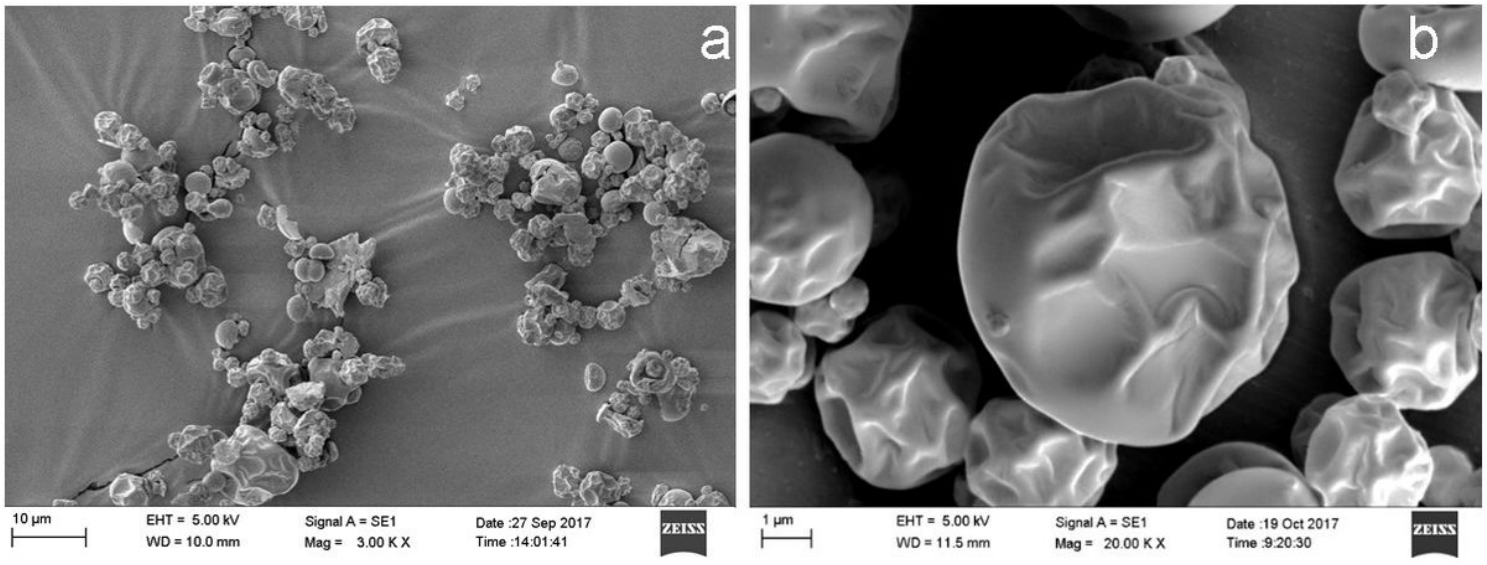

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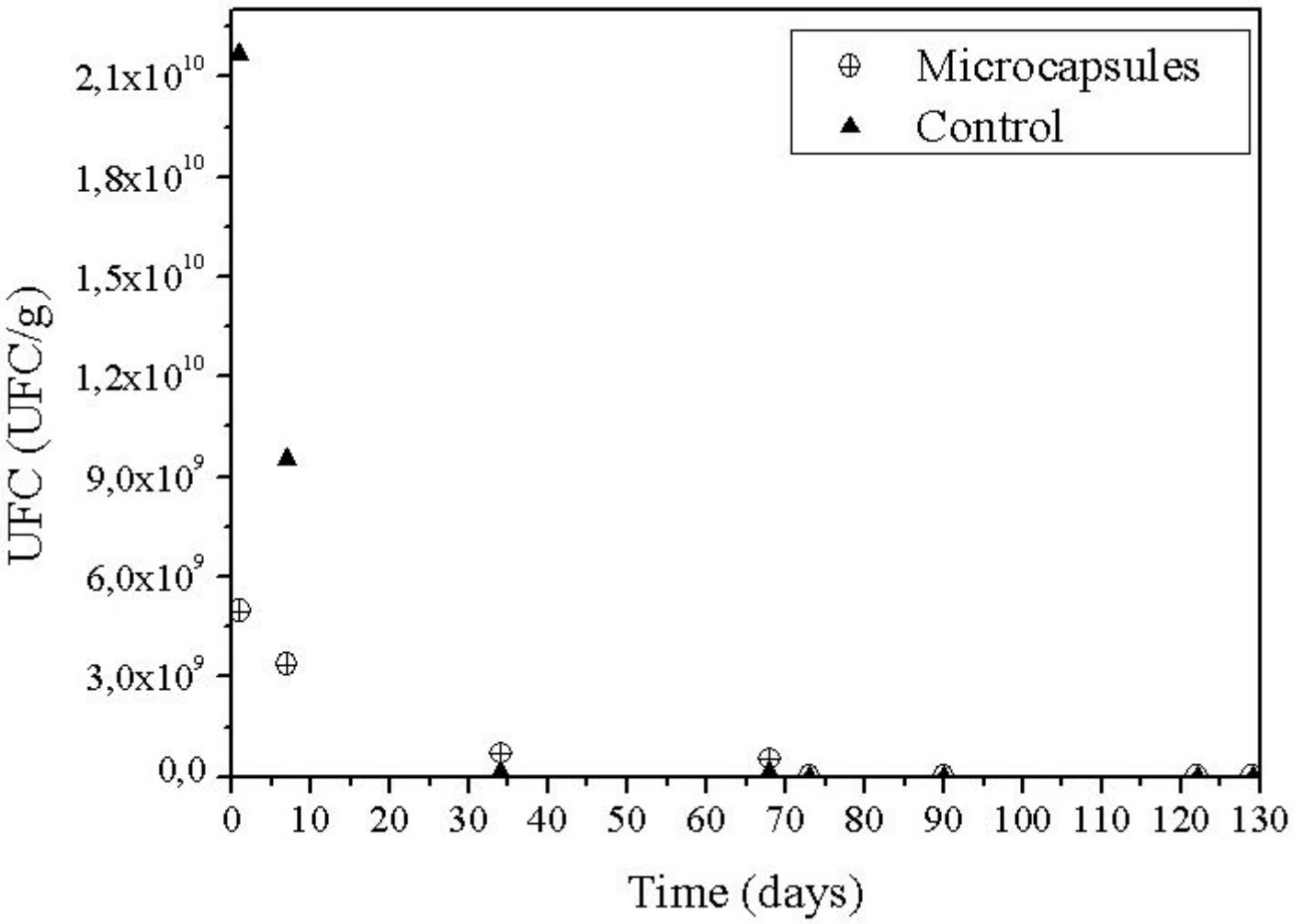

Figure 4

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