Evaluation of the microencapsulation process of conidia of \textit{Trichoderma asperellum} by spray drying

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Abstract

Microencapsulation of microorganisms has been studied to increase product shelf life and stability to enable the application in sustainable agriculture. In this study, the microencapsulation of \textit{Trichoderma asperellum} conidia by spray drying (SD) was evaluated. The objective was to assess the effect of drying air temperature and wall material (maltodextrin DE20, MD20) concentration on the microencapsulation and to identify the optimum conditions to produce, in high yield, microparticles with low moisture, high conidial viability and conidial survival. Microparticles were characterized in terms of morphology, particle size, and shelf life. A central composite rotatable design (CCRD) was used to evaluate the effect of operating parameters on drying yield (DY), moisture content, conidial viability (CV), and conidial survival (SP). Microencapsulation experiments were carried out under optimum conditions to validate the obtained model. The optimum temperature and MD20/conidia dry weight ratios were 80 °C and 1:4.5, respectively, which afforded a drying yield of 63.85 ± 0.86\%, moisture content of 4.92 ± 0.07\%, conidial viability of 87.10 ± 1.16\%, and 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. The mathematical models accurately predicted all the variables studied, and the association of the microencapsulation technique using DE20 maltodextrin was able to optimize the process and increase the product’s shelf life. It was also concluded that high inlet air temperatures negatively affected conidia survival, especially above 100 °C.

Keywords Microparticle · Powder · Wall material · Central rotational compound design · Biological control

Introduction

Sustainable agronomy and environmental protection are issues in constant discussion around the world and very important nowadays [1, 2, 3, 4, 5]. Fungi of the genus \textit{Trichoderma} spp. show potential for plant growth promotion [6, 7] and are the main species used for biological control of phytopathogens. \textit{Trichoderma} can interact with phytopathogens through different mechanisms, such as parasitism, antibiosis, and competition, and have a resistance-inducing effect on plants against diseases [8, 9, 10].

Compared with chemical agents, \textit{Trichoderma} products have some disadvantages, particularly with respect to shelf life and viability during storage and field application [11, 12, 13]. High water activity, high drying temperatures during processing [11], prolonged storage, high storage temperatures [12, 14, 15], contamination of growth medium, and oxidative stress [15] are factors that affect the viability of fungal formulations.

Microencapsulation has been extensively studied to protect microorganism cells and increase their survival [16, 17, 18]. Microencapsulation by spray dryer (SD) is studied to produce microbial powder formulations [16]. During spray drying, product temperature is kept low by the rapid evaporation of water from droplets, which makes the technique suitable for drying heat-sensitive products [16, 17, 18] without compromising quality. Spray drying has been shown to increase the shelf life of fungal preparations [11, 12, 14,
15, 17, 18]. 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 [19, 20, 21].

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 [18, 22, 23, 24, 25, 26, 27]. 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 [28].

In this context, a central composite rotatable design (CCRD) was used as a statistical tool to support the optimization hypothesis under the evaluated conditions of spray-dryer drying of conidia of *Trichoderma asperellum* (URM 5911), ensuring your survival and its stability. The objectives of this work were to evaluate the influence of drying air temperature and maltodextrin concentration on the characteristics of *T. asperellum* conidial powders produced by microencapsulation 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

**Trichoderma asperellum** conidial suspensions and wall material

*Trichoderma asperellum* (URM 5911) 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 spore concentration, germination, and colony forming unit (CFU) count prior to each experiment. The spore suspension, with an average concentration of 2.5 × 10⁸ spores/mL was stored at 5 °C until the moment of the process.

**Microencapsulation by spray drying**

The *Trichoderma 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 × 10⁴ L h⁻¹, and atomization air flow rate of 2400 L h⁻¹.

To optimize process conditions, we used a central composite rotatable design (CCRD), with three replicates at the center point and 11 runs 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.

Variables and their levels are presented in Table 1. The upper and lower limits of independent variables were determined according to preliminary tests and previous studies on microencapsulation of *Trichoderma* sp. conidia [11, 12].

To perform the microbiological analyses, the powders were rehydrated up to the same spore concentration of the initial solution fed to the dryer and characterized for germination and colony forming units (CFUs), with the main objective of evaluating the amount of viable conidia (CV) and percentage of conidia (SP) survival.

### Process optimization and experimental validation

Optimum operational conditions for microencapsulation of *T. asperellum* conidia by spray drying were determined by response surface methodology (RSM) based on CCRD, as described by Box [27]. Model equations were imported into MATLAB, contour plots were constructed and superimposed, and the region of optimum response for all variables was identified.

### Physicochemical, microbiological, and morphological analyses

Microbiological analyses were performed using powders rehydrated to the initial spore concentration of the feed solution. Germination percentage was determined to assess conidial viability, and CFU counts were used as a measure of conidial survival.

*Trichoderma asperellum* conidial suspensions were characterized for spore concentration, germination and colony forming units (CFUs), with the main objective of evaluating the amount of viable conidia (CV) and percentage of conidia (SP) survival.

### Table 1 Central composite rotatable design for spray-drying microencapsulation of *T. asperellum* conidia using maltodextrin DE20 as wall material

| Factor | −1.41 | −1 | 0 | 1 | 1.41 |
|--------|-------|----|---|---|-----|
| Ti (°C) | 51.8 | 60 | 80 | 100 | 108.2 |
| Concentration [Ta/MD20] | 1:0 | 1:1.4 | 1:4.9 | 1:8.4 | 1:9.8 |

*Ta*, drying air inlet temperature; concentration [Ta/MD20], dry mass of conidia of *T. asperellum* by dry mass of maltodextrin DE20.
percentage, and CFU count. Microparticles were characterized for moisture content [29], spore concentration, germination percentage, and CFU count.

Conidia were counted using a Neubauer chamber. Conidial viability after spray drying was determined using the germination test proposed by Danielson and Davey [30] and Milner [31], with modifications. CFU counting was carried out according to the method of Jin and Custis [11]. Viable conidia concentration was expressed as CFU per gram. Conidial survival percentage was calculated as the CFU count of the feed solution divided by the CFU count of spray-dried powders times 100 [32].

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).

Trichoderma 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 [15].

### 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 and discussion

#### Effects of experimental conditions on microparticles

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.

Drying yields ranged from 36.5% (run 5) to 76.37% (run 6) (Table 2). Zhou [33] 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.

Due to the significant variability inherent in bioprocesses, a significance level of 90% was assumed. Equations 1, 2, 3, and 4 were obtained for the model with coded variables based on the t-student test represented in Table 3.

Equation 1 ($R^2 = 0.7$) describes the drying yield as a function of the significant ($p < 0.10$) variable:

$$DY(\%) = 61.00 + 9.69T_i$$

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

The drying yield response surface plots were plotted as a function of inlet air temperature and maltodextrin...
concentration (Fig. 1a). The increase in the inlet air temperature had a positive effect on the drying yield, observed in the Eq. 1. Thus, run 5, which was performed at the lowest temperature (51.8 °C), resulted in the lowest yield (36.50%).

In the work of LeClair [34], 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) [26] 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.

Runs 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 run 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:

\[
\text{Moisture} = 3.78 - 1.75T_i
\]  

where \(T_i\) is the inlet air temperature.

The increase in inlet air temperature caused a significant decrease in microparticle moisture content (Fig. 1b). The coefficient of determination \(R^2\) for the response variable

### Table 3 Statistical results of central composite rotatable design: effects of the factors of microencapsulation process of T. asperellum conidia by spray drying

| Factor | Effect | p-level | t-student | Effect | p-level | t-student | Effect | p-level | t-student | Effect | p-level | t-student |
|--------|--------|---------|-----------|---------|---------|-----------|---------|---------|-----------|---------|---------|-----------|
| Mean   | 60.99  | 0.000   | 12.789    | 3.77    | 0.000   | 7.321     | 83.75   | 0.000   | 10.560    | 81.87   | 0.000   | 13.507    |
| \(T_i\) | 19.38  | 0.021   | 3.313     | -3.50   | 0.002   | -5.544    | -70.51  | 0.000   | -7.248    | -64.49  | 0.000   | -8.674    |
| \(T_i^2\) | -3.67  | 0.621   | -0.525    | 1.17    | 0.182   | 1.548     | -46.45  | 0.010   | -4.000    | -44.75  | 0.004   | -5.044    |
| \(T_a\) | 2.80   | 0.652   | -0.478    | -0.32   | 0.629   | -0.513    | 8.78    | 0.408   | 0.902     | 5.61    | 0.484   | 0.754     |
| \(T_a^2\) | 3.71   | 0.618   | -0.531    | 0.53    | 0.511   | 0.707     | -12.46  | 0.332   | -1.073    | -11.85  | 0.239   | -1.335    |
| \(T_i:T_a\) | -1.89  | 0.828   | -0.228    | 1.34    | 0.193   | 1.501     | -1.71   | 0.906   | -0.124    | -10.38  | 0.368   | -0.989    |

\(T_i\), drying air inlet temperature; \(T_a\), dry mass of T. asperellum conidia by dry mass of MD20; \(DY\), drying yield; \(CV\), viable conidia; \(SP\), percentage of survival.

Fig. 1 Response surfaces of the CCRD results as a function of the drying air inlet temperature and MD20 concentration in relation to: a drying yield, b moisture of the microparticles, c viable conidia, and d percentage of survival of the conidia of Trichoderma asperellum.
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. The effect of maltodextrin concentration on microparticle moisture was not significant (p > 0.10).

Jin and Custis [11] investigated the microencapsulation of *Trichoderma 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 problem was observed in the present study at an inlet temperature of 51.8 °C (run 5). At high temperatures, however, no problems were observed, in accordance with the reported by Jin and Custis [11]. High inlet air temperatures (100.00 and 108.20 °C in runs 3, 4, and 6) negatively influenced conidial viability and survival (Table 2). 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. [17], the survival of microencapsulated *Bacillus 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 *T. asperellum* found in the present study. Decrease in conidial viability occurred mainly because of the increase in temperature, as seen by Eq. (3).

\[
CV(\%) = 83.75 - 35.25T_i - 23.227_i^2
\]

(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.

Jin and Custis [11] reported that the lowest *T. harzianum* conidial survival was obtained at an inlet air temperature of 140 °C and the highest, at 60 °C.

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

\[
CS(\%) = 81.81 - 32.247T_i - 22.387_i^2
\]

(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) [11] showed that the best conidial survival results were obtained using inlet air temperatures of 50–80 °C, whereas the worst results were obtained using temperatures of 120–140 °C.

The response surface plot of conidial survival (Fig. 1d) revealed that inlet air temperatures of 60 to 70 °C and wall material/conidia dry weight ratios of 1:5 to 1:9 gave the highest conidial survival percentages.

The use of high temperatures in the drying process contributes to a more significant heat and mass transfer gradient, that is, greater evaporation of the solution and, consequently, lower moisture in the final product and higher process yields. However, as seen in the results of this study and in the literature, when it comes to drying biological products, care must be taken because the higher yield may mean more quantity of dry product (mass), but not necessarily greater viabili- ity of microorganisms. In a previous study by this group Braga et al. [35], it was found that the thermosensitivity of *T. asperellum* occurs from 90 °C, which was proven with cell membrane disruption in scanning electron microscopy images. That is, for industrial processes in which higher temperatures are required, there is a need to use protective agents to protect and enable that microorganism; even so, it may affect its viability.

### Optimization of process conditions and validation of the model

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

![Figure 2: Contour curves for the percentage of moisture, viable conidia, and percentage of survival of the conidia of *Trichoderma asperellum* obtained by the CCRD](image)
The optimum responses were achieved with inlet air temperatures of 77 to 79 °C and wall material/conidia dry weight ratios of 1:3.8 to 1:7 (Fig. 2). An inlet air temperature of 80 °C was chosen as the optimum temperature, as it was within the operating range of the spray dryer used. The optimum wall material/conidia dry weight ratio was defined as 1:4.5. New tests were carried out using these parameters to confirm the results and validate the model. Equations (1–4) were used to calculate the predicted response values. The goodness of fit of the models was evaluated by the coefficient of determination ($R^2$) and residue analysis. The predicted and experimental data used to validate the models are in the Table 4.

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

**Morphology of microencapsulated Trichoderma asperellum conidia**

The scanning electron microscopy (SEM) images of *T. asperellum* conidia microencapsulated showed that a dense and robust structure surrounded *T. asperellum conidia* (Fig. 3) corroborating previous results observed by the group [35]. Most particles had an irregular matrix structure with a wrinkled appearance and concave depressions. In this group’s previous work [35], different wall materials were used (sucrose, maltodextrin, gum arabic, whey powder, and lactose) for the microencapsulation of *T. asperellum*. In general, it was observed that the particles presented irregular matrix formation, of indefinite shape, with deformed and toothed particles with varied concavities and sizes, with the greater or lesser intensity of these characteristics according to the wall material used [35]. Another essential point to consider about the morphology is the drying process and the process conditions used. According to Lian [36] and Favaro-Trindade [37], concave shapes are characteristic of atomized particles. According to Rodriguez-Huezo [38], the formation of dimples is due to the drying temperature used. Lian [36] reported that wall material affects dimple size. The morphology of the microparticles obtained in the present work is similar to that of microcapsules of *T. harzianum* conidia obtained by Muñoz-Celaya [12] by spray drying using maltodextrin DE10 at 20% (w/v) as wall material. Ma et al. [17] produced microcapsules with 100% (w/v) maltodextrin concentration and spray-drying conditions (air-inlet temperature of 145 °C, feed flow rate of 550 mL h$^{-1}$. The scanning electron microscopy of *B. subtilis* microcapsules presented by the authors was very similar to the *T. asperellum* particles (Fig. 3). These literature data contribute to the thesis that the particle’s morphology is more influenced by the microcapsule formation (type of wall material and drying process) than the microencapsulated microorganism (nucleus). Another point that can be observed is that,

| Table 4 | Comparison of results of drying yield, moisture, viable conidia, and survival of *T. asperellum conidia*, predicted versus experimental results for model validation using an inlet air temperature of 80 °C and a maltodextrin/conidia dry weight ratio of 1:4.5 |
|---------|---------------------------------------------------------------------------------|
| Drying yield (%) | Moisture (%) | Viable conidia (%) | Survival percentage (%) |
| Pred | Exp | Pred | Exp | Pred | Exp | Pred | Exp |
| 61.13 | 63.85 $\pm$ 0.86 | 3.8 | 4.92 $\pm$ 0.07 | 83.1 | 87.10 $\pm$ 1.16 | 81.41 | 85.78 $\pm$ 2.88 |

*Pred.*, predicted value; *Exp.*, experimental value

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Fig. 3 Scanning electron microscopy (SEM) of microparticles containing *Trichoderma asperellum* conidia produced under optimum spray drying conditions: a 10 μm scale bar (9.00 KX approximation) and b 1 μm scale bar (approximation of 20.00 KX)
apparently, the amount of wall material also does not exert much influence on the morphological characteristics of microencapsulated cells.

As conidia were well covered by the wall material and as an intermediate inlet air temperature (80 °C) was used, good results of conidial viability (87.10 ± 1.16%) and conidial survival (85.78 ± 2.88%) were obtained. Jin and Custis [11] and Muñoz-Celaya [12] obtained conidial survival percentages of 76% and 86% using sucrose and maltodextrin as wall material, respectively, for the microencapsulation of *T. harzianum*.

**Particle size distribution of *Trichoderma asperellum* conidia microparticles**

The particle size distribution of *T. asperellum* conidia microparticles obtained using optimum parameters is evaluated in Table 5.

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 (Table 4). Particle size distribution was similar to that obtained by Jin and Custis [11], 10–25 μm, and Ma et al. [17], 7–14 μm, who used the spray-drying technique to obtain microencapsulated *T. harzianum* and *B. subtilis*, respectively. According to Ma et al. [17], the survival rate of *B. subtilis* B99-2 decreased with the decreasing microparticle size. This hypothesis of the authors makes sense first because larger particles can contain more than one cell, which facilitates the protection of the nucleus. In addition, larger particles will have smaller contact areas, which results in higher resistance to heat transfer or the action of other agents that can decrease cell viability. It is vital to highlight that particle size is greatly influenced by the solution and the process conditions used in the spray dryer. In other words, the size of the atomized droplet varies directly with the viscosity of the emulsion at a constant atomization rate. The higher the viscosity of the emulsion, the larger the droplets formed during atomization, which influences the particles’ size and shape [39].

**Viability of *Trichoderma 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 × 10⁹ to 3.37 × 10⁹ CFU g⁻¹ compared with 2.17 × 10¹⁰ to 9.53 × 10⁹ 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 (Fig. 4). Microencapsulation proved to be an option to preserve *T. asperellum* at room temperature, since *Trichoderma* spp. are widely used in agriculture as biological control agent, mainly in nematode control [6].

The CFU count of microencapsulated conidia (5.33 × 10⁸ CFU g⁻¹) and control (1.50 × 10⁸ CFU g⁻¹) at 68 days of storage was significantly lower than the initial count, and conidial survival was 10.74 ± 1.16% and 0.69 ± 0.61%, respectively. After 129 days of storage, the number of viable conidia was significantly lower in both samples, 2.70 × 10⁶ CFU g⁻¹ for microencapsulated conidia and 8.67 × 10⁶ CFU g⁻¹ for the control. According to Harmen and Custis (2015) [40], it is ideal that *Trichoderma* formulations contain 5 × 10⁹ CFU g⁻¹ to be effective in a variety of applications. According to a study by EFSA [41], however, the minimum number of viable *Trichoderma* spp. conidia can vary depending on the type of application and crop.

In Muñoz-Celaya et al. [12], the viability of spray-dried *T. harzianum* conidia without wall material decreased

![Fig. 4](image-url) CFU count of microencapsulated *Trichoderma asperellum* conidia and the control sample throughout storage at 29 °C

### Table 5

| Exp | Tᵢ (°C) | [Ta/MD20] | d0.1 (µm) | d0.5 (µm) | d0.9 (µm) |
|-----|---------|-----------|-----------|-----------|-----------|
| Control | 80 | 1:0       | 2.15      | 4.40      | 9.52      |
| Microcapsules (validation) | 80 | 1:4.5     | 1.56      | 5.78      | 11.76     |

Exp., experiment; Tᵢ, drying air inlet temperature; Ta, conidia of *T. asperellum*; MD20, maltodextrin DE20
significantly after 4 weeks of storage at 4 and 29 °C. However, when using maltodextrin DE10 and gum Arabic as wall materials, conidial survival decreased only after 8 weeks of storage (conidial survival percentages of 40% and 23% at 4 °C and 29 °C, respectively). Other studies evaluating the survival of spray-dried microorganisms at different storage temperatures showed that microorganisms remained more stable under refrigeration [15, 42, 43]. Domingues et al. [44] 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. [45] 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 [46,47]. Chávez and Ledeboer [23] also reported high cell viability using low oxygen levels during storage of probiotic microorganisms.

Muñoz-Celaya et al. [12] 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.

**Conclusion**

It was concluded that the spray drying microencapsulation process improved the viability of *T. asperellum* conidia and increased its stability during storage compared to the control in the conditions used in this study. The drying process conditions and wall material can affect the morphological and physical characteristics of *T. asperellum* microencapsulated. In addition, the process temperature was fundamental to maintaining the spores’ viability and stability. The mathematical model validated the drying air temperature at 80 °C as ideal. Under optimum inlet air temperature and maltodextrin concentration conditions, conidial viability and survival were 87.10 ± 1.16% and 85.78 ± 2.88%, respectively.

**Author contribution** All authors whose names appear on the submission made substantial contributions to the conception or design of the work, drafted the work or revised it critically for important intellectual content, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy of any part of the work.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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

**Declarations**

**Competing interests** The authors declare no competing interests.

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