

Bioformulation of *Meyerozyma guilliermondii* LMA-Cp01 microencapsulated in soy protein isolate-sodium alginate: characterization and control of blue mold on Persian lime

Bioformulación de *Meyerozyma guilliermondii* LMA-Cp01 microencapsulada en aislado proteico de soya-alginato de sodio: caracterización y control del moho azul en limón Persa

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ABSTRACT

Biocontrol agents have been shown to be an effective alternative to chemical fungicides. Nevertheless, the main difficulties in the usage of these biocontrol agents are the development of suitable formulations and delivery systems to be applied in commercial conditions. This work aimed to evaluate the storage stability, physicochemical and rehydration properties, and biocontrol efficacy against *Penicillium italicum* on Persian lime of *Meyerozyma guilliermondii* microencapsulated in soy protein isolate-sodium alginate by spray-drying process. The bioformulation viability was 10.2 Log CFU g⁻¹; however, its viability decreased after 120 d storage to 6.8 (4°C) and 5.5 Log CFU g⁻¹ (25°C). Desirable physicochemical values of the bioformulation were obtained. Low humidity (5.7 %), *A_w* (0.29) and hygroscopicity (8.2 %), and high dispersibility values (94 %) were observed. The *M. guilliermondii* bioformulation effectively reduced the incidence (50 %) and severity (46 %) of blue mold on Persian lime, as well as the fresh cells of this yeast. FTIR, DSC, and TGA analysis revealed interactions of wall materials. The *M. guilliermondii* bioformulation can be used as an effective alternative to chemical fungicides for the management of postharvest diseases in citrus fruit.

KEY WORDS: *Prunus* Bioformulation; *Meyerozyma guilliermondii*; physicochemical properties; bioefficacy; Persian lime.

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RESUMEN

Los agentes de biocontrol son una alternativa eficaz a los fungicidas químicos. El desarrollo de formulaciones y sistemas de entrega adecuados para el uso comercial son los principales desafíos. Este trabajo tuvo como objetivo evaluar la estabilidad de almacenamiento, las propiedades fisicoquímicas y de rehidratación, y la eficacia de biocontrol contra *Penicillium italicum* en limón Persa de *Meyerozyma guilliermondii* microencapsulada en aislado proteico de soya-alginato de sodio mediante el proceso de secado por aspersión. La viabilidad de la bioformulación fue de 10.2 Log CFU g⁻¹; sin embargo, su viabilidad disminuyó durante 120 días de almacenamiento a 6.8 (4 °C) y 5.5 Log CFU g⁻¹ (25 °C). Se obtuvieron valores de los parámetros fisicoquímicos deseables de la bioformulación. Se observaron bajos valores de humedad (5.7 %), *A_w* (0.29) e higroscopicidad (8.2 %), y altos valores de dispersabilidad (94 %). La bioformulación de *M. guilliermondii* así como las células frescas de la levadura, redujeron eficazmente la incidencia (50 %) y severidad (46 %) del moho azul en limón Persa. Los análisis FTIR, DSC y TGA revelaron interacciones de los materiales de la pared. La bioformulación de *M. guilliermondii* puede utilizarse como un método eficaz para el manejo de enfermedades poscosecha en cítricos, como alternativa a los fungicidas químicos.

PALABRAS CLAVE: *Bioformulación; Meyerozyma guilliermondii; propiedades fisicoquímicas; bioeficacia; limón persa.*

Introduction

Citrus is a major fruit crop grown worldwide (Wang *et al.*, 2021). Mexico is an important global producer of lemons; however, this fruit is prone to fungal decay at harvest (Vázquez-Vázquez *et al.*, 2021). Blue mold caused by *Penicillium italicum* is one of the main citrus diseases that cause economic losses (González-Estrada *et al.*, 2018). The use of synthetic fungicides to manage fungal diseases may have harmful effects on human health and the environment (González-Gutiérrez *et al.*, 2023; Moraes Bazioli *et al.*, 2019). Biological control of fungal diseases in fruits and vegetables, particularly the application of antagonistic yeasts, has been proven to be an effective alternative to chemical fungicides (Moraes Bazioli *et al.*, 2019).

Nevertheless, acceptance and widespread use of biocontrol products remain limited, likely because of their shortcomings when applied commercially (Droby *et al.*, 2016). The most

important challenge in using these biocontrol products is developing suitable formulations and delivery systems (Hubballi et al., 2023). Therefore, handling the improvement of biocontrol agents (BCA) under commercial conditions is necessary (Usall et al., 2016). One way to achieve this goal is to develop and package a formulation that maintains proper shelf-stability, distribution, and application in the agricultural market (Carbó et al., 2019; Gotor-Vila et al., 2019). The effectiveness of a BCA is based on its ability to survive under commercial conditions and colonize the surfaces of fruits and vegetable crops. The microencapsulation process may protect the BCA from hostile conditions. Spray drying has been employed to preserve BCAs in a dry form, offering the advantage of drying large volumes of cultures quickly and at a low cost (Aguirre-Güitrón et al., 2018). With the aim of biocontrolling phytopathogenic fungi, a bioformulation based on soy protein isolate and sodium alginate has been developed, encapsulating the yeast *Meyerozyma guilliermondii* LMA-Cp01 by spray drying (López-Cruz et al., 2020). The yeast *M. guilliermondii* is an effective BCA against several fungal diseases on different fruits (Agirman & Erten, 2020; López-Cruz et al., 2020, 2023; Sun et al., 2021). For industrial purposes, controlling the release and complete rehydration in water of these microencapsulated BCAs is important for practical use and is often described as an essential quality attribute. Also, physicochemical studies indicate whether the wall materials are suitable for efficient encapsulation (Ji et al., 2016). The characterization of microencapsulated *M. guilliermondii* is crucial to improve the formulation and increase its potential for future commercial applications.

Therefore, this study aimed to evaluate the thermal and physicochemical properties, shelf-life, and effectiveness against *P. italicum* on Persian lime of the spray-dried *M. guilliermondii* LMA-Cp01 bioformulation.

Materials and Methods

Antagonistic yeast

M. guilliermondii strain LMA-Cp01 belongs to the collection of the Technological Institute of Tepic, Mexico. Stock cultures cryo-preserved in 40 % glycerol (v/v) at -80 °C were subcultured on nutrient yeast dextrose agar plates [NYDA, which contained nutrient broth (8 g L⁻¹), yeast extract (5 g L⁻¹), dextrose (10 g L⁻¹), and agar (15 g L⁻¹)] at 25 °C for 48 h. Yeast biomass was produced in a LiFlux-GX fermenter (BIOTRON, Korea) with 2 L of culture media constituted by sugar cane molasses (40 g L⁻¹) and soy protein isolate (5 g L⁻¹), incubated at 28 °C, 280 rpm, for 36 h, starting from an initial concentration of 10⁶ CFU mL⁻¹. The biomass was recovered by centrifugation (9400 xg, 25 °C, 5 min).

Bioformulation by spray drying

Bioformulation of *M. guilliermondii* was produced by the spray-drying method according to the optimum conditions reported in a previous work (López-Cruz et al., 2020). Briefly, the yeast biomass recovered after centrifugation (10 g biomass/ 100 mL of suspension) was mixed with the

wall materials SPI (3.8 %, w/v) and sodium alginate (0.06 %, w/v), previously autoclaved. Then, the suspension was fed to a mini spray dryer B-290 (BUCHI, Flawil, Switzerland) with a feed flow of 3 mL min⁻¹, at 113 °C inlet air temperature. The flow rate and air pressure conditions were 35 m³ h⁻¹ and 0.6 MPa, respectively. The feed suspensions were kept in constant stirring at 150 rpm at 25 ± 1 °C.

Viability and storage stability

3 g of bioformulation sample were packed into microtubes, sealed with parafilm, and stored at two temperature conditions (4 °C and 25 °C). To determine viable cells of microencapsulated *M. guilliermondii*, 0.1 g of powder was suspended in 9.9 mL of 0.85 % (w/v) NaCl solution, vortexed for 1 min, and set aside for 9 min. Viable cells were enumerated on NYDA by the dilution plate technique (incubated at 28 °C/ 48 h). The viability of microencapsulated *M. guilliermondii* was evaluated every 30 days for 120 days.

Physicochemical analysis

Moisture content

The moisture content of the spray-dried sample was determined using a thermobalance (Sartorius MA 35). Two grams of the powder sample were placed on an aluminum plate and dried at 105 °C until the moisture content reached a constant value.

Water activity

Water activity was determined in an Aqualab 4TEV (Decagon devices) hygrometer. Samples of 5 g were placed inside the chamber, where the water activity was determined by the dew point principle.

Solubility

One gram of powder was rehydrated in 100 mL of distilled water, stirred at 600 rpm until the entire sample was solubilized, and then centrifuged at 2,598 g for 5 min. An aliquot of 2 mL of supernatant was taken and placed in Petri dishes. Finally, it was dried on a stove at 105 °C for 5 hours (Fritzen-Freire *et al.*, 2012). The solubility was calculated by Equation 1.

$$Solubility (\%) = \frac{Mf}{M0} * 100 \quad (Equation 1)$$

Where *M0*: initial dry mass of sample (g), *Mf*: final dry mass of sample (g)

Wettability

One gram of powder was placed over the surface of 100 mL of distilled water without agitation. Wettability was expressed as the time required for 1 g of powder to disappear from the water surface (Fuchs et al., 2006).

Dispersion in water

The dispersion in water of the microencapsulated *M. guilliermondii* components was analyzed by measuring changes in transmittance with a spectrophotometer set a $\lambda=700$ nm. Samples of 0.1 g were rehydrated in 20 mL of distilled water, shaken at 600 rpm for 1 min, and then rehydrated for another 1 min in static conditions (Gotor-Vila et al., 2019). The first measurement was done after 5 s of agitation with three consecutive assessments. A second measurement was done after 1 min in static conditions with three consecutive assessments. The rate of dispersion was calculated based on Equation 2:

$$\text{Dispersibility (\%)} = \left(1 - \frac{t_2 - t_1}{t_2}\right) * 100 \quad (\text{Equation 2})$$

Where t_1 is the measurement after agitation and t_2 is the measurement after leaving in static for 1 min. The experiment was repeated three times.

Hygroscopicity

Five g of powder was placed in a desiccator at 21 °C and set at 76 % relative humidity (solution of 36 g of NaCl in 100 g of water) on a petri dish. The weight was recorded every 15 min until it reached a constant value. The results were reported in grams of water absorbed by 100 g of dried solid ($\text{g } 100 \text{ g}^{-1}$). The absorbed water percentage was calculated using Equation 3:

$$\text{Higroscopicity (\%)} = \frac{\Delta m / (M + M_i)}{1 + \Delta m / M} \quad (\text{Equation 3})$$

Where Δm (g) is the increase in weight of powder after equilibrium, M is the initial mass of powder, and M_i is the free water content of the powder before exposure to the humid air environment.

Fourier Transform Infrared Spectroscopy (FTIR)

Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) (Thermo Scientific Nicolet, iS5 iD5, Waltham, USA) was used to evaluate sodium alginate, SPI, empty microcapsules (mixture of SPI-sodium alginate), free cells, and microencapsulated *M. guilliermondii*. The samples were placed onto the ATR crystal, and all the spectra were recorded from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} .

Thermogravimetric analysis (TGA)

TGA was performed from 25 to 600 °C under a nitrogen atmosphere (20 mL min^{-1}) using a TGA 550 thermogravimetric analyzer (TA Instruments, New Castle, USA). Samples (5–10 mg) were analyzed in a platinum pan at a heating rate of 5 °C min^{-1} . The temperature at which samples start to degrade was assumed as the onset temperature of the second weight loss step, the first one being assigned to moisture loss. The TGA curves were obtained using the software TRIOS 5.0.0.44616 (TA Instruments, New Castle, USA). TGA analysis was carried out with sodium alginate, SPI, empty microcapsules, free cells, and microencapsulated *M. guilliermondii*.

Differential scanning calorimetry analysis (DSC)

The DSC curves were obtained using a DSC 250 calorimeter (TA Instruments, New Castle, USA) heated from 30 °C to 300 °C. Samples of ~5 mg of powder were analyzed in aluminum sealed pans at a heating rate of 5 °C min^{-1} . The DSC equipment was preliminarily calibrated with a standard reference of indium. DSC analysis was carried out with sodium alginate, SPI, empty microcapsules, and microencapsulated *M. guilliermondii*.

Efficacy of *M. guilliermondii* biofungicide against *P. italicum* on Persian lime

The efficacy of microencapsulated and 36-hour-old fresh cells of *M. guilliermondii* was evaluated against *P. italicum* on Persian lime (*Citrus latifolia* Tanaka). Fruits were washed with tap water, disinfected with 2 % (v/v) sodium hypochlorite for two minutes, rinsed with sterile tap water, and allowed to dry. Then, they were wounded in the equator with a nail to produce an injury (3 mm in diameter $\times$ 3 mm in depth). Treatments at a concentration of 10^8 CFU mL^{-1} were applied preventively by spraying the fruits with a handheld sprayer. Sterile water was applied as a control treatment. Once the treatments dried (2 hours later), 15 μL of the pathogen *P. italicum* at 10^5 conidia mL^{-1} was added to each wound. Then, the fruits were allowed to dry at room temperature and incubated at 25 °C and 90 % RH. After 10 days, the incidence reported as the number of infected fruits and the severity reported as rot lesion diameters were measured. Ten fruits constituted a replicate, and each treatment was replicated four times.

Statistical analysis

The data analysis was performed using STATISTICA 12.0 software (StatSoft Inc., Tulsa,

USA). Analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$) among the data. Differences between means were detected using LSD Fisher's test.

Results and Discussion

Viability and storage stability of microencapsulated *M. guilliermondii*

The effect of two storage temperatures on the viability of microencapsulated *M. guilliermondii* was evaluated (Figure 1). The spray-dried formulation had an initial viable cell concentration of $10.2 \text{ Log CFU g}^{-1}$. Since the effectiveness of a biocontrol product is related to its viability, it is important to minimize cell death during the formulation process and to ensure minimal loss of formulated product viability during storage. The viability of microencapsulated *M. guilliermondii* tended to decrease ($p < 0.05$) during storage. At 4°C , almost 93 % ($9.4 \text{ Log CFU g}^{-1}$) of the microencapsulated *M. guilliermondii* remained viable after a month and 85.5 % ($8.7 \text{ Log CFU g}^{-1}$) after 2 months. Viable cells declined to 7.84 and $6.9 \text{ Log CFU g}^{-1}$ at the end of 90 and 120 days, respectively. After a month at 25°C , viability of 86.3 % ($8.7 \text{ Log CFU g}^{-1}$) of the microencapsulated *M. guilliermondii* was observed. After 90 and 120 days of storage, viable cells declined to 6.5 and $5.5 \text{ Log CFU g}^{-1}$, respectively.

In general, samples stored at 4°C showed higher viable cell counts ($p < 0.05$) than those stored at 25°C . Palazzini et al. (2020) reported similar trends, with spray-dried formulations stored at 4°C maintaining higher viable cell counts than those stored at 25°C . A low storage temperature is a condition reported to minimize the metabolic rate of the BCAs and prevent the accumulation of toxic metabolites and depletion of nutrients, thereby prolonging the life span of microorganisms (Melin et al., 2011). The minimum requirement for the application of biopesticide-formulated products in agriculture is 10^6 CFU g^{-1} (Mulatu et al., 2021). Result of this study demonstrated that microencapsulated *M. guilliermondii* achieved a retention of viable cells exceeding this requirement after four months of storage at 4°C . However, the storage stability of commercial microbial bioformulations can be expected to last between six months and one year (Berninger et al., 2018). Hence, further research is needed to enhance storage stability of microencapsulated *M. guilliermondii*.

The viability loss over time may be attributed to the oxygen in the air in contact with stored cells (Aguirre-Güitrón et al., 2018). Vacuum packaging could be an attractive option for maintaining an adequate shelf life of bioproducts. It may diminish the oxygen in the package atmosphere, minimizing the rates of chemical and biochemical reactions (Elzein et al., 2009).

Physicochemical properties of *M. guilliermondii* bioformulation

Moisture content and water activity

Values of moisture content and water activity of the *M. guilliermondii* bioformulation are shown in Table 1. Similar results to this work were reported by Aguirre-Güitrón et al. (2018) with

a spray-dried formulation of *M. caribbica*, where 5 ± 0.37 % of moisture content and A_w of 0.33 ± 0.11 were found. By contrast, [Carbó et al. \(2017\)](#) reported moisture content values from 10.3 to 15.2 % of some fluidized-bed drying formulations using potato starch as the carrier, different concentrations of protectants, and inlet air temperatures from 40 to 60 °C. The wall materials and drying conditions could affect the encapsulant's moisture content and water activity. Successful survival of formulated microorganisms requires A_w values below 0.3, which contributes to microbiological stability during storage because there is less water available for biochemical reactions ([Chávez & Ledebøer, 2007](#)) and to prevent powder agglomeration ([Ho et al., 2022](#)).

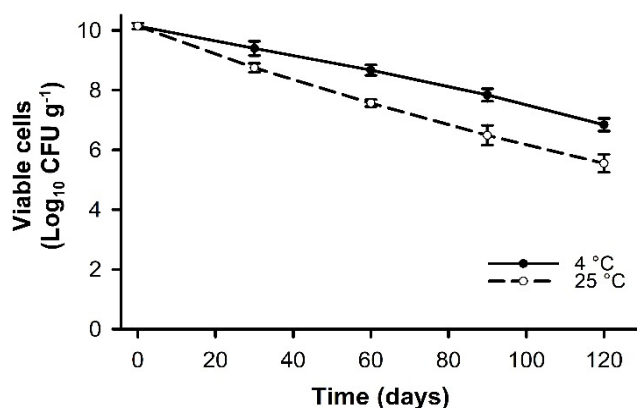


Figure 1. Stability of microencapsulated *M. guilliermondii* under two storage conditions. Error bars represent the standard deviation of the mean.

Source: Own elaboration based on results.

Table 1. Physicochemical properties of *M. guilliermondii* biofungicide.

Property	Value
Moisture (g 100 g ⁻¹)	5.7 ± 0.6
Water activity	0.3 ± 0.1
Hygroscopicity (g 100 g ⁻¹)	8.2 ± 0.4
Wettability (min)	15 ± 6
Solubility (g 100 g ⁻¹)	33 ± 4
Dispersibility (%)	94 ± 4

Values are the mean ± standard deviation.

Wettability

The wettability time of the formulation was found to be high (Table 1). [Arepally et al. \(2020\)](#) reported wettability times from 1.9 to 5.9 min for spray-dried encapsulated probiotics in Arabic gum and maltodextrin. Wettability is the ability of a powder to be penetrated by a liquid due to capillary forces. This property depends largely on pore size, moisture content, and the hydrophilic nature of the wall materials ([Hogekamp & Schubert, 2003](#)). The prolonged wettability time, generally undesirable in instant powders, becomes an advantageous functional characteristic in this application by facilitating the gradual release of the biocontrol agent, which serves as a protective barrier. Then, SPI used to encapsulate *M. guilliermondii* was chosen as a wall material for its ability to control the release of the antagonistic yeast due to slow hydration in water.

Hygroscopicity

The hygroscopicity value of microencapsulated *M. guilliermondii* is presented in Table 1. [Arepally et al. \(2020\)](#) reported hygroscopicity values higher than those found in this study. They reported values ranging from 12 to 21.15 g 100 g⁻¹ dry weight of a spray-dried encapsulated probiotic using gum Arabic as wall material. Hygroscopicity is mainly affected by the composition, type, and concentration of carrier material used for encapsulation ([Suhag et al., 2016](#)). High-molecular-weight solutes are less hygroscopic and help reduce the final powder's hygroscopicity. Generally, a powder with low hygroscopicity, low water content, and a degree of clumping is considered a good powder ([Bhusari et al., 2014](#)).

Solubility and dispersion in water

M. guilliermondii microcapsules were low soluble in water (Table 1). This is due to the SPI used as a wall material, which has more hydrophobic compounds exposed on its surface. However, its low solubility is advantageous for encapsulated BCA products intended for preharvest and/or postharvest application, as a protective barrier against environmental conditions is desired ([Rathore et al., 2013](#)).

The dispersion rate of microencapsulated *M. guilliermondii* was higher (Table 1). On the contrary, SPI showed low dispersibility (33.3 %), while sodium alginate showed the highest dispersibility (>99 %). Nevertheless, the mixture of wall materials of SPI-sodium alginate (empty microcapsules) showed a higher value of dispersibility (96.3 %). Moreover, no statistical differences ($p < 0.05$) between empty microcapsules and microencapsulated *M. guilliermondii* were observed. [Gotor-Vila et al. \(2019\)](#) reported precipitation rates ranging between 0.9 and 2 % and 31.1 to 43 % for two fluid-bed spray-dried formulations of *Bacillus amyloliquefaciens* in maltodextrin and potato starch, respectively.

Factors influencing these properties include particle size and density, porosity, and surface properties, among others. It should also be noted that particle dispersion in the aqueous phase is not the same as dissolution. Instead, achieving complete dispersion of the insoluble particles is

the most relevant quality index for biocontrol products ([Shittu & Lawal, 2007](#)).

FTIR analysis

A peak at 1027 cm^{-1} corresponding to the stretching of C-O bonds of sodium alginate FTIR was observed (Figure 2a). SPI showed peaks at 1635 cm^{-1} (Amide I) and 1520 cm^{-1} (Amide II), corresponding to C=O stretching and C-N deformation vibrations, respectively (Figure 2b). These peaks are characteristic of proteins and peptides. A peak at 1235 cm^{-1} (Amide III) depicts a C-N stretching of amide bonds. The empty microcapsules of SPI-sodium alginate spectra (Figure 2c) exhibited an increase in the intensity of amide I and II groups compared to the SPI due to the amino groups of SPI interacting with the carboxyl groups of sodium alginate to form a complex containing an amide bond ([Albano et al., 2019](#)).

Overall, empty microcapsules (Figure 2c) and microencapsulated *M. guilliermondii* (Figure 2e) exhibited similar behavior, with the only differences observed in the intensity of the peaks. These results evidenced weak interactions between *M. guilliermondii* cells and wall materials, indicating that the yeast may be released from the microcapsules.

Thermal analysis

TGA

Two representative stages were differentiated by the thermogravimetric curves for sodium alginate, SPI, empty microcapsules, and microencapsulated *M. guilliermondii* (Figure 3). The first mass loss of the TGA curves refers to moisture loss (between 30 and $100\text{ }^{\circ}\text{C}$). Above this temperature range, the second mass loss is associated with the decomposition process. During this stage, decomposition reactions may occur in the components of the microcapsules, i.e., proteins and carbohydrates ([Fritzen-Freire et al., 2012](#); [Rivera-Aguilar et al., 2020](#)). Sodium alginate, SPI, and empty microcapsules showed onset temperatures of 215 , 250 , and $247\text{ }^{\circ}\text{C}$, respectively. Moreover, [Miss-Zacarias et al. \(2022\)](#) reported that thermal degradation of sodium alginate occurred between 185.5 and $474\text{ }^{\circ}\text{C}$, as observed in this study.

On the other hand, free cells of *M. guilliermondii* showed three stages of mass variation; the second and third stages were observed at onset temperatures of 261 and $375\text{ }^{\circ}\text{C}$, respectively, corresponding to the decomposition of sugars, proteins, and complex carbohydrates. Microencapsulated *M. guilliermondii* showed an onset decomposition temperature of $233\text{ }^{\circ}\text{C}$; its thermogram did not show the third stage of mass variation, indicating that there were no free cells around microcapsules. Then, it can be inferred as an adequate microencapsulation process.

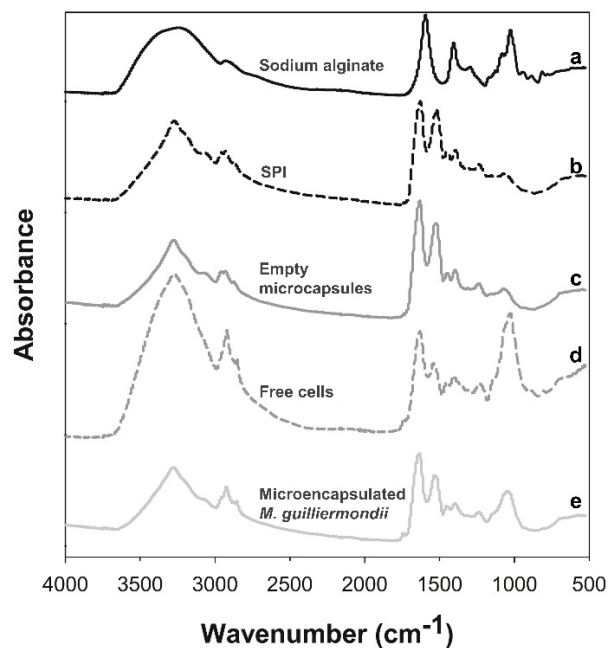


Figure 2. FTIR spectra of microencapsulated *M. guilliermondii* and its components. Sodium alginate (a), SPI (b), empty microcapsules (c), free cells (d), and microencapsulated *M. guilliermondii* (e).

Source: Own elaboration based on results.

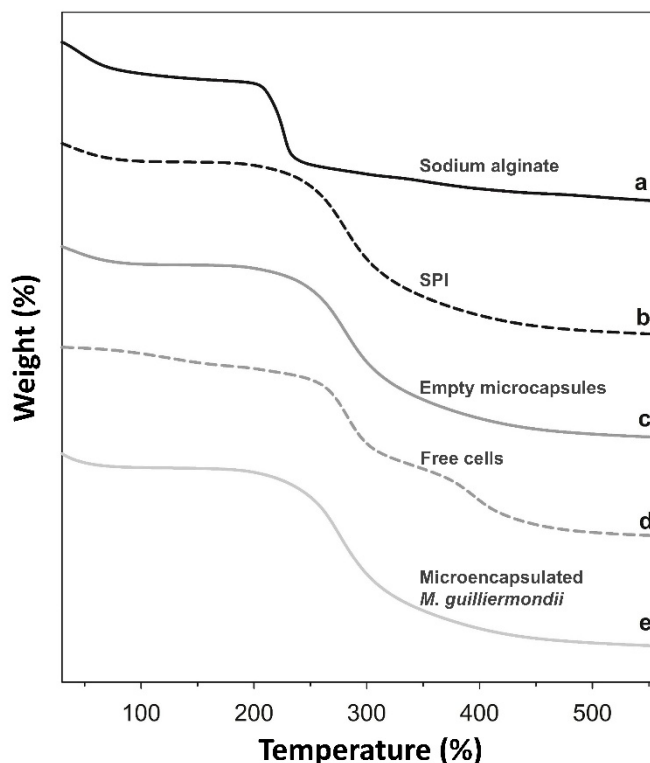


Figure 3. Thermogravimetric profile of the *M. guilliermondii* bioformulation components. Sodium alginate (a), SPI (b), empty microcapsules (c), free cells (d) and, microencapsulated *M. guilliermondii* (e).

Source: Own elaboration based on results.

DSC

For all samples, the DSC curves showed two well-defined endothermic peaks corresponding to the melting points (Figure 4). An additional thermal event (exothermic peak) was observed in sodium alginate, which can be attributed to the oxidation of decomposition remnants. Separately, sodium alginate and SPI showed Tg values of 132 and 134.3 °C, respectively. A Tg value of 139.4 °C was observed for the SPI-sodium alginate mixture. These data indicate a possible cross-linking between the polymers, as described by [Albano et al. \(2019\)](#), which may improve the physicochemical and thermal properties of the two macromolecules, as evidenced by the FTIR analysis (Figure 2). The DSC curve of microencapsulated *M. guilliermondii* closely resembled that of the empty microcapsules. The *M. guilliermondii* addition did not affect the Tg value of microcapsules. The weak interactions of the polymeric material with the yeast cells, evidenced in the FTIR spectra (Figure 2), were confirmed in the DSC analysis.

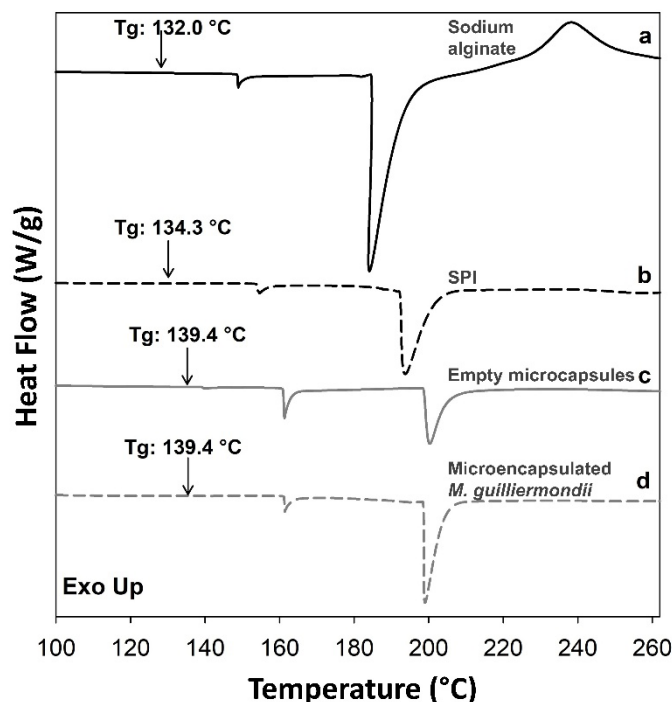


Figure 4. DSC curves of the *M. guilliermondii* bioformulation components. Sodium alginate (a), SPI (b), empty microcapsules (c) and, microencapsulated *M. guilliermondii* (d).

Source: Own elaboration based on results.

Biocontrol efficacy of microencapsulated *M. guilliermondii* against *P. italicum* on Persian lime

The biocontrol efficacy of the spray-dried formulation of *M. guilliermondii* LMA-Cp01 was tested on Persian lime (Figure 5). Both treatments of *M. guilliermondii*: formulated or fresh cells were effective ($p < 0.05$) in reducing the incidence (Figure 5a) and severity (Figure 5b) of the infections caused by *P. italicum* on Persian lime compared to the control. No significant differences in severity and incidence were observed among treatments of *M. guilliermondii*. Incidence reductions were 33 ± 13 and 50 ± 13 % when fresh cells and microcapsules were used, respectively. Severity reductions were 40 ± 22 % for fresh cells and 46 ± 11 % for microcapsules of *M. guilliermondii*. Similar results of the efficacy of another *M. guilliermondii* strain against *P. italicum* on Persian Lime were reported by Wang et al. (2021). Nevertheless, a few studies of the use of this yeast as a biocontrol of citrus diseases have been reported. *M. guilliermondii*

LMA-Cp01 is an effective biocontrol agent to manage several phytopathogenic fungi such as *C. gloeosporioides*, *P. digitatum*, *P. italicum*, *P. expansum*, *Rhizopus stolonifer*, *Botrytis cinerea*, *Lasiodiplodia theobromae*, *Neofusicoccum batangarum*, and *Cladosporium cladosporioides* (Ayón-Macias *et al.*, 2023; Covarrubias-Rivera *et al.*, 2024; López-Cruz *et al.*, 2023, 2025). Thus, the possibility of controlling the main postharvest pathogens of citrus on Persian lime could extend its application.

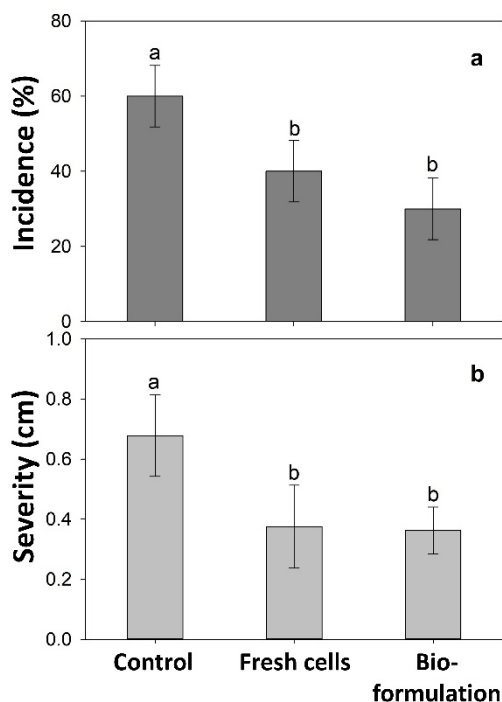


Figure 5. Efficacy of *M. guilliermondii* bioformulation against *P. italicum* on Persian lime. Disease incidence (a) and severity (b) evaluated after 10 days of storage at 25 °C and 90 % RH. Different letters indicate significant differences ($p < 0.05$) according to Fisher's LSD test. The bars represent the means of 10 fruits x 4 replicates and the error bars represent the standard deviation of the means.

Source: Own elaboration based on results.

Conclusions

The spray-drying microencapsulation process enabled the development of a biocontrol formulation based on *M. guilliermondii* in SPI-sodium alginate with desirable physicochemical properties. FTIR and thermal analysis evidenced interactions between SPI and sodium alginate,

as well as weak interactions between *M. guilliermondii* and wall materials. These findings suggest increased stability of *M. guilliermondii*, as well as an adequate microencapsulation process that ensures progressive delivery. Moreover, microencapsulated *M. guilliermondii* is a promising formulation to manage blue mold caused by *P. italicum* on Persian lime. Nonetheless, further research is needed to improve the storage stability of the bioformulation. In addition, it is necessary to evaluate the effectiveness of the doses and application frequencies of the bioformulation under commercial field or packing plant conditions.

Author contributions

Conceptualization (MCS, JARS); Investigation (RLC), Methodology (RLC, MCS, JARS), Supervision (MCS, JARS), Validation (MCS), Funding acquisition (MCS), Writing - review & editing (RLC, MCS, JARS), Visualization (MCS), Data curation (RLC), Formal analysis (RLC), Project administration (MCS). All authors of this manuscript have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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