



Scalable Citral and Lemongrass Essential Oil Nanocapsules for Gray Mold Control in Grapes

Vinícius Bertoncello Molon¹ · Manuela Pires Onzi¹ · Bruna Spido Vieira¹ · Fernando Joel Scariot² · Ana Paula Longaray Delamare² · Thiago Barcellos¹

Received: 13 April 2026 / Accepted: 29 May 2026 / Published online: 4 June 2026
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Abstract

Gray mold is a serious economic-impact fungal disease caused by *Botrytis cinerea* that threatens the quality and storage life of a wide range of fruits and vegetables. Currently, pathogen control relies heavily on synthetic fungicides, raising environmental and health concerns. This study explores the development and evaluation of poly(lactic acid) (PLA) nanocapsules loaded with citral, a natural compound found in plant essential oils that has been shown to have antifungal properties. PLA-encapsulated lemongrass essential oil (LGEO) was also evaluated as a natural citral-containing substance. The nanoencapsulated systems were successfully formulated in 2 L batches, yielding stable colloidal suspensions with mean diameters from 220 to 224 nm and encapsulation efficiencies from 84 to 87%. The antifungal activity was first assessed by in vitro conidial germination assays, which showed complete inhibition was achieved at concentrations $\geq 0.05\%$ (v/v) for both encapsulated and non-encapsulated citral and LGEO. Subsequently, the effectiveness of the nanoencapsulated citral and LGEO in preventing gray mold was evaluated using *Vitis vinifera* grapes inoculated with *B. cinerea*. The results revealed that nanoencapsulation proved remarkably superior: citral- and LGEO-loaded nanocapsules reduced lesion diameters by up to 72% and 63%, respectively, after 168 h of storage. In contrast, free citral and LGEO showed limited efficacy against gray mold. These findings highlighted the potential of nanoencapsulation as a strategy to enhance the efficacy and safety of bioactive terpenes as alternative, sustainable fungicides for postharvest disease management.

Keywords *Botrytis cinerea* · Phytopathogenic fungi · Postharvest control · Nanotechnology · Poly(lactic acid)

Introduction

Ensuring global food security is an increasingly critical challenge as the world's population is projected to reach 9.7 billion by 2050 (UN, 2022). To meet this demand, agricultural production must not only increase but also become more efficient at reducing postharvest losses. Currently, approximately 13.3% of the world's food is lost between the harvest and retail stages (FAO, 2022). Fruits and vegetables are particularly vulnerable, with fungal pathogens accounting

for 20% to 25% of total losses (Sharma et al., 2009; Taye et al., 2023). Among fungal pathogens, *Botrytis cinerea*, the causative agent of gray mold, is one of the most destructive fungi that can decimate yields and compromise grape quality (Dean et al., 2012; Rahman et al., 2024; Williamson et al., 2007). Traditionally, the control of postharvest diseases relied on the application of synthetic fungicides. However, the continuous and indiscriminate use has led to significant concerns such as emergence of multidrug-resistant fungal strains (Ramos et al., 2025). Furthermore, increasing consumer awareness regarding pesticide residues in food and the detrimental effects of these compounds on human health and the environment have sparked a global demand for safer and sustainable alternatives (Beyuo et al., 2024).

In this context, citral, a mixture of the isomers' geranial and neral, is a well-known monoterpenic aldehyde widely reported for its strong antifungal activity, particularly through mechanisms involving membrane disruption and interference with ergosterol biosynthesis (Pedroso et al.,

✉ Thiago Barcellos
thiago.barcellos@ucs.br

¹ Biotechnology Laboratory for Natural and Synthetic Products, University of Caxias Do Sul, Francisco Getúlio Vargas 1130, Caxias Do Sul, RS 95070-560, Brazil

² Enology and Applied Microbiology Laboratory, University of Caxias Do Sul, Francisco Getúlio Vargas 1130, Caxias Do Sul, RS 95070-560, Brazil

2024). Due to these properties, citral has emerged as a promising natural compound for postharvest quality control (Bin Duan et al., 2024). While the efficacy of citral is well documented, its practical application faces the same drawbacks associated with citral-rich naturally occurring essential oils, such as high volatility, sensitivity to environmental degradation, and potential (Solon et al., 2025). Lemongrass essential oil (LGEO) stands out as the primary biological source of this aldehyde, frequently containing citral levels exceeding 70% of its total composition (Khasanah et al., 2025). This high concentration makes LGEO a natural, cost-effective, and readily available alternative to the isolated compound (Balanay & Guinancias, 2025).

Essential oils (EOs) and their major compounds have emerged as an alternative to synthetic pesticides. These compounds, synthesized by plants, possess broad-spectrum antimicrobial, insecticidal, and antioxidant properties (El Khetabi et al., 2022). Most EOs are classified as “generally recognized as safe” (GRAS), making them candidates for postharvest applications where direct food contact is common (Jackson-Davis et al., 2023). Lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) essential oil (LGEO) is a particularly noteworthy natural and abundant source of citral. LGEO and citral have demonstrated potent inhibitory effects against a variety of phytopathogens (Camero et al., 2019; Fontana et al., 2021; Hirsch et al., 2024). Moreover, lemongrass is a resilient perennial crop that offers high oil yields, ensuring a sustainable and consistent supply for industrial applications (Balanay & Guinancias, 2025; Silva et al., 2023).

Despite their potential, the direct application of EOs, or even their bioactive main compounds, in agriculture and food handling is limited, as they are sensitive to environmental factors such as light, oxygen, and temperature, which lead to degradation and loss of bioactivity (Reyes-Jurado et al., 2020). Their volatility also means that their protective effects are often short-lived. Furthermore, essential oils may present important limitations for postharvest applications, as they can cause phytotoxic effects at the concentrations required for effective fungal control and, due to their strong and characteristic aroma, may also alter the natural sensory profile of treated food products, potentially compromising consumer acceptance (Falleh et al., 2020; Nazzaro et al., 2017).

Nanoencapsulation offers a solution to many of these challenges by incorporating labile compounds into polymeric nanostructures. Therefore, shielding them from environmental degradation, reducing their volatility, and achieving controlled release of the bioactive compounds over time. Previous research has shown that poly(lactic acid) (PLA) nanocapsules can effectively deliver LGEO to control fungi such as *Colletotrichum gloeosporioides* in apples, thereby significantly reducing the phytotoxicity compared to the free oil (Antonioli et al., 2020). PLA is an ideal polymer for this

purpose, as it is biocompatible, biodegradable, and derived from renewable resources (Wi et al., 2025).

A major bottleneck in the field of nanotechnology is the “scale-up” process. Studies on encapsulation of EOs have successfully shown the development at the laboratory scale (typically 50–100 mL). However, studies showing whether the scaling up of these systems maintains their physico-chemical properties, such as particle size, polydispersity, and encapsulation efficiency, are limited (Ephrem et al., 2014). For nanoencapsulated fungicides to be commercially viable, they must be reproducible at scales that meet agricultural demands. Additionally, there is a pressing need for more in vivo studies to validate the efficacy of these systems in real-world postharvest conditions (El Khetabi et al., 2022).

This work focused on optimizing the formulations of the PLA-nanoencapsulated biofungicide citral and the PLA-nanoencapsulated lemongrass essential oil, which is a citral-rich natural source. The study demonstrated the feasibility of scaling up the production process to 2 L batches. The antifungal activity was evaluated through in vitro conidia germination assays and in vivo preventive treatments on postharvest grapes. The results provide a comprehensive assessment of the nanoencapsulated systems as a sustainable alternative for controlling gray mold on postharvest fruits.

Materials and Methods

Chemicals

Acetone of analytical grade was purchased from Dinâmica (Indaiatuba, Brazil). Citral (95%, Lot#STBG4262V) and sorbitan monostearate (Span 60, Lot#SLCN2059) were obtained from Sigma-Aldrich (St. Louis, USA). HPLC grade acetonitrile purchased from Merck (Darmstadt, Germany) and Polysorbate 80 (Tween 80, Lot#AUTO045242) from DEG Produtos Químicos (Jardim Oriental, Brazil). The poly (lactic acid) (NatureWorks® Ingeo™ Biopolymer 4060D) was kindly donated by Cargill®-Brazil, São Paulo. PDA (Potato Dextrose Agar, Lot#VM1078630-411) was obtained from Merck-Millipore (Burlington, USA). All commercial reagents were used without prior purification. Deionized water was obtained by a water purification system that produced ultra-pure water type I grade.

Extraction and Characterization of Lemongrass Essential Oil

LGEO was extracted by steam distillation from dried young and mature leaves. The plant material was harvested in September 2023 (spring season in Brazil) from cultivated plants at the experimental field of the University of Caxias do Sul (29°08'28.9" S, 50°59'29.3" W). The citral and essential oil

chemical composition was determined by gas chromatography with a flame ionization detector (GC-FID), and by gas chromatography-mass spectrometry (GC-MS) analyses. The GC-FID analyses were performed at a gas chromatograph (Shimadzu 2010 Plus, Kyoto, Japan) equipped with a cross-linked (5% phenyl)-methylpolysiloxane HP-5 capillary column (30 m × 0.32 mm internal diameter (i.d.), 0.25-μm film thickness; Hewlett Packard, USA). The oven temperature program was initially 40 °C for 8 min, then increased to 180 °C at a rate of 3 °C min⁻¹, then increased to 230 °C at 20 °C min⁻¹, and maintained at 230 °C for 10 min. The injector and detector temperatures were set at 250 °C. Hydrogen was used as the carrier gas at a pressure of 34 kPa, with a split ratio of 1:50. A 1.0-μL sample diluted in hexane (1:10) was injected (Antonioli et al., 2020). The GC-MS analysis was conducted on an HP 6890 gas chromatograph coupled with an HP 5973 mass selective detector (MSD). The analysis used the same HP-5 capillary column and oven temperature program as described for GC-FID analyses. The interface temperature was 280 °C, with a split ratio of 1:100. Helium served as the carrier gas at 56 kPa, with a flow rate of 1.0 mL min⁻¹. Ionization was performed at 70 eV. A 1.0-μL sample (1:10 in hexane) was injected (Antonioli et al., 2020). Identification of the essential oil constituents was based on a comparison of their mass spectra with those in the Wiley 275 MS library and their linear retention indices (LRIs), calculated relative to a series of *n*-alkanes (C9–C22), with published values. The relative abundance of each compound was determined from the peak areas obtained in the GC-FID chromatogram. As complementary analyses, LGEO and citral density were determined using a portable densimeter (DMA-35n, Anton Paar) at 25 °C.

Polymer-Oil Core Swelling Compatibility

The swelling interaction of the PLA with citral and LGEO was determined by the weighing method (Guterres et al., 2000). The PLA films were obtained by placing PLA pellets between two aluminum plates. The plates were pressed at 100 °C for 5 min under a load of five tons using a hydraulic press (PHS-15t, Schulz, Brazil). After pressing, the films were cooled in a second hydraulic press (P10 ST, Bove-nau, Brazil), maintaining the same load until they reached room temperature. The resulting films were cut into 10 mm × 5 mm rectangular pieces. The initial mass of each sample was recorded, and then each sample was immersed in citral or LGEO (1 mL) in amber glass vials. The vials were sealed and stored at room temperature. The samples were retrieved at the following time intervals: 0, 3, 5, 10, 15, 30, and 45 days. At each time point, the samples were removed from the solution, dried on absorbent paper, and weighed to determine mass changes.

Optimized Procedure for PLA Nanocapsules Containing Lemongrass Essential Oil or Citral

Nanocapsule preparation was carried out by interfacial deposition of a preformed polymer through solvent displacement, as described by Fessi et al. (1989), with some adjustments. The organic phase was prepared by dissolving PLA (100 mg) and sorbitan monostearate (Span 60, 77 mg) in 30 mL of acetone at 40 °C. Separately, the aqueous phase was prepared by dissolving polysorbate 80 (Tween 80; 77 mg) in 50 mL of deionized water at room temperature. Both phases were stirred for 1 h before the incorporation of citral or LGEO (100–500 μL) as the active agent added to the organic phase under vigorous stirring and maintained for 10 min. Subsequently, the organic phase was introduced into the aqueous phase using an automatic dosing pump (OFA 40D, OFA Ambiental, Brazil) at a constant flow rate of 0.5 mL min⁻¹. The suspension was stirred for an additional 10 min, followed by solvent removal under reduced pressure at 40 °C, yielding a final volume of 50 mL of citral (Citral-NC) or LGEO nanocapsules (LGEO-NC).

Batch Scale-up Formulation of Nanocapsules

In a 5-L glass jar, 4.0 g of PLA and 3.08 g of sorbitan monostearate (Span 60, 0.10% m/v) were dissolved in 1.2 L of acetone at 40 °C. The resulting mixture was kept under magnetic stirring for 1 h until complete dissolution. After, the temperature was reduced to 30 °C, and 20.0 mL of the active core compound (citral or LGEO) was added, keeping the resulting mixture under stirring for another hour. This organic solution was injected at a flow-rate of 0.5 mL min⁻¹ through an automatic dosing pump (OFA 40D, OFA Ambiental, Brazil) into an aqueous solution, composed of 3.08 g of polysorbate 80 (Tween 80, 0.10% m/v) diluted in 2.0 L of deionized water in a 5-L glass jar equipped with a six-hole paddle stirring shaft at 250 rpm. At the end of the addition, the resulting suspension was stirred for an additional 1 h. Finally, the acetone was removed by evaporation. In this stage, an evaporation system was set up with an external water bath heated at 40 °C and slightly reduced pressure at 800 mbar, for acetone removal and recovery.

Dynamic Light Scattering and Zeta Potential

Particle size distribution, expressed as hydrodynamic mean diameter, was determined by dynamic light scattering (DLS) using a ZetaSizer Pro-Blue (Malvern Instruments Ltd., Malvern, UK) under controlled conditions (25 °C), considering spherical geometry and a refractive index of 1.459 (PLA). The zeta potential was assessed

using the same analyzer. DTS0012 and DTS1070 cuvettes were used for particle size and zeta potential measurements, respectively. All measurements were recorded and analyzed using ZS Xplorer software (v. 3.3.1.5, Malvern Instruments Ltd., Malvern, UK).

Encapsulation Efficiency and Loading Capacity

The encapsulation efficiency (EE) of the nanocapsules was assessed by HPLC using a Shimadzu chromatographic system (Kyoto, Japan) equipped with dual LC–20AD pumps and an SPD–20A UV detector operating at 240 nm. Analyses were performed on a Lichrospher RP18 reverse-phase column (250 × 4.6 mm, 5 µm) thermostated at 30 °C, using acetonitrile–water (60:40, v/v) as the mobile phase at a flow rate of 0.5 mL min^{−1}. The analysis time was 25 min, and the injection volume was 5 µL, and the quantification was based on citral, used as an external standard, selected due to its predominance in LGEO. A calibration curve was prepared in acetonitrile with ten concentration points (0.0020–0.1000% v/v), analyzed in triplicate, yielding the equation $y = 418,932,864.22x + 458,817.01$ and a correlation coefficient of 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.0015% and 0.0047% (v/v), respectively.

Quantification of citral entrapped in LGEO–NC and Citral–NC was performed by ultrafiltration. Briefly, 200 µL of the nanocapsule suspension was placed in a centrifugal filter device (Ultrafree®-MC, 10 kDa; Millipore) and centrifuged at 10,000 rpm for 10 min. The retained phase was recovered, solubilized in acetonitrile (1 mL), and passed through 0.2-µm nylon syringe filters before HPLC determination. The EE was calculated as the ratio between the amount of citral or LGEO initially added and the encapsulated citral content quantified by HPLC, according to the Eq. (1).

$$EE(\%) = \left(100 - \frac{(C_i - C_f)}{C_i} \right) \times 100 \quad (1)$$

where C_i , the added citral (% v/v) in the formulation, and C_f , the concentration of citral (% v/v) retained in the ultrafiltrate nanocapsules. For the LGEO, the obtained EE was divided by 0.7493, based on the citral composition of 74.93% in the LGEO.

The loading capacity (LC) was calculated based on the obtained encapsulation efficiency, according to the Eq. (2).

$$LC(\%) = \frac{m_e}{(m_e + m_p)} \times 100 \quad (2)$$

where m_e , the mass of encapsulated citral or LGEO, and m_p , the mass of the PLA.

Morphological Analysis of the Prepared Nanocapsules

Morphological characterization of the nanocapsules was performed by FEG–SEM using a TESCAN MIRA 3 instrument (Tescan, Brno, Czech Republic) equipped with a Schottky field-emission gun and operated at 10 kV. Samples were prepared by airbrushing the nanocapsule suspension onto 10 mm² silicon plates and drying in a desiccator. Gold sputter coating was applied before image acquisition, and particle size measurements were obtained using ImageJ software (v. 1.53i, NIH, USA).

Fungal Isolates Identification and Conidia Suspension Preparation

B. cinerea (MH665643.1) was isolated from infected grapevines cultivated in “Serra Gaúcha” wine region located in Southern Brazil. To obtain the fungal conidia used in the in vitro and in vivo assays, the potato dextrose agar (PDA) plates inoculated with *B. cinerea* were incubated for 14 days at 25 °C, and under a 12-h photoperiod. After 14 days, a brush was used to scrub the plate, removing hyphae, and the plate was then kept for an additional 24 h at 25 °C under a 12-h photoperiod. The plates were then washed with 5 mL of sterile distilled water. The fungal conidia were quantified using a Neubauer chamber and adjusted to a final concentration of 1×10^5 conidia mL^{−1}.

In Vitro Conidia Germination Inhibition Assay

A conidial germination assay was performed to assess the antifungal activity of the Citral–NC–5 and LGEO–NC–5 at seven different concentrations (0.4–0.006% v/v) prepared by serial dilution and tested against *B. cinerea*. Non-encapsulated citral or LGEO emulsions containing Tween 80 (0.5% w/v) were used for comparison, while the Tween 80 solution alone served as the control. Each well of a 96-well microplate received 90 µL of the test solution and 10 µL of a conidial suspension adjusted to 1×10^5 conidia mL^{−1}. The plates were incubated for 48 h at 25 °C under a 12-h light/dark cycle. Experiments were conducted in duplicate. Conidial germination was evaluated by inverted light microscopy (VT series, Meiji Techno Co., Japan) at 400× magnification. A germination scale previously established by Molon et al. (2026) was employed. Scale **I** comprises observable hyphal growth, where filamentous vegetative hyphae extend across the substrate, indicating that the treatment did not inhibit germination. Scale **II** is characterized by the presence of germ tubes without further elongation, suggesting that the treatment may restrict polarized growth. And finally, scale

III consists of conidia with no visible emergence, indicating complete suppression of germination by the treatment (Fig. 1).

Flow Cytometry

The *B. cinerea* conidia were harvested from 14-day-old PDA plates and subsequently purified by filtration. Spores were meticulously washed with sterilized water, and the concentration was subsequently adjusted to 1×10^5 spores mL^{-1} in Synthetic Defined (SD) medium [0.67% Yeast Nitrogen Base (Y1250, Merck, Darmstadt, Germany), 2% glucose, pH 6.8]. The conidia suspension treated with encapsulated or non-encapsulated LGEO or citral in the concentrations of 0.05% and 0.1% (v/v), and the untreated conidia were incubated for 6 h in an orbital shaker (150 rpm) at 28 °C. Esterase activity and membrane integrity were evaluated by flow cytometry using a FACSCalibur flow cytometer (Becton–Dickinson Biosciences, USA) equipped with an argon-ion laser emitting at 488 nm. Flow cytometry data from 10,000 cells were acquired using CellQuest Pro software v.5.2 (BD Bioscience, USA), and the data analysis was performed using FlowJo v.10 software (TreeStar, Inc., USA). Cell membrane integrity and esterase activity were determined using the FungaLight Yeast™ CFDA, AM/Propidium Iodide Vitality kit (Invitrogen, Waltham, USA) that includes 5-carboxyfluorescein diacetate (5-CFDA) to determine esterase activity.

In Vivo Antifungal Assay in Postharvest Grapes

An in vivo assay was conducted on Thompson Seedless grapes (*Vitis vinifera* L.) to determine the antifungal efficacy of Citral–NC–5 and LGEO–NC–5 formulations. These assays included the encapsulated and non-encapsulated citral and LGEO at concentrations that were previously determined in in vitro assays. Healthy and uniform berries were selected and disinfected with a solution of 70% ethanol, then with a 0.5% sodium hypochlorite solution, and finally rinsed with sterile water. A Tween 80 solution (0.5%, w/v) was used as a non-inoculated control. Fourteen berries were used for each treatment. Each berry was wounded (~1-mm

depth), treated with 10 μL of the respective formulation, and subsequently inoculated with 10 μL of a *B. cinerea* conidial suspension (1×10^5 conidia mL^{-1}), except for the control group, both aliquots were dispensed directly into the wound using a micropipette. Wounded but untreated grapes were included as a negative control. Samples were stored in sterile plastic boxes at 24 ± 1 °C with 95% relative humidity for 7 days. Disease development was monitored by measuring lesion diameters in two perpendicular directions at 96, 128, and 168 h using a digital caliper.

Statistical Analysis

Data normality and homogeneity of variance were verified using the Shapiro–Wilk and Levene tests, respectively. Once these assumptions were met, data were analyzed by one-way ANOVA, and means were compared using Tukey's test. The threshold for statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS 22.0 (IBM Analytics, Tulsa, USA), and graphs were generated using Origin 2018 (OriginLab, USA).

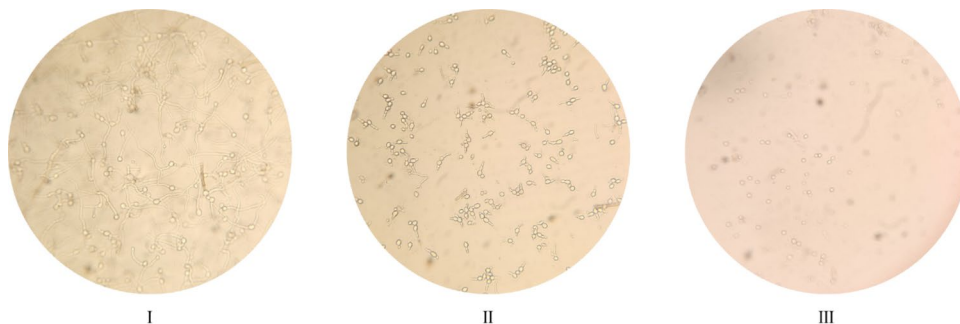
Results and Discussion

Lemongrass Essential Oil and Citral Chemical Characterization

Gas chromatography coupled with mass spectrometry (GC–MS) was employed to evaluate the quality and chemical composition of extracted LGEO and the commercially available citral. The identification of the compounds was based on molecular ion fragmentation patterns and supplemented by comparing the obtained mass spectra with reference library data. To improve the accuracy of compound identification, linear retention indices (LRIs) were calculated for each component and cross-referenced with literature values.

Fifteen compounds were identified for the LGEO, accounting for 94.0% of the registered chromatographic peaks (Table S1). Citral, which is a mixture of the

Fig. 1 The three stages of the germination scale of the fungus *B. cinerea* under a microscope (microscopic images at 400 $\times$ taken from test-wells after 24 h)



stereoisomers neral and geranial, is the primary component of LGEO, accounting for 74.93% of its composition, reinforcing its role as the primary bioactive compound. Neral and geranial were present in the essential oil at 31.53% and 43.40%, respectively, with a geranial-to-neral ratio of 1.38. The third main constituent of LGEO was β -myrcene, present at 13.29%.

These values align with findings reported by other researchers for LGEO cultivated in Brazil, where citral contents of 67.4% and 59.84% were reported, both studies indicating a predominance of geranial (Antonioli et al., 2020; Cruz et al., 2023). The chromatographic analysis of the commercial citral revealed only the peaks for neral and geranial, as expected (chromatogram shown in Fig. S2). It exhibited a geranial-neral ratio of 1.44, which is close to the ratio in the LGEO. Regarding relative density, LGEO presented a value of 0.9034 ± 0.0001 , while citral exhibited a value of 0.8908 ± 0.0002 .

Polymer-Oil Core Swelling Compatibility

In the development of nanoencapsulated systems, a critical consideration is ensuring compatibility between the wall material and the core components. If the core substance can dissolve the wall material, the result will likely be a nanoemulsion rather than true nanocapsules (Ruy et al., 2011). In our previous work, we identified adequate compatibility between LGEO and PLA, with a mass increase of 30–35% in the first 5 days, followed by no mass variation for the remaining 55 days of analysis (Antonioli et al., 2020). In this study, however, compatibility was remeasured despite the slight variation in the LGEO composition. The initial swelling of the PLA plates in contact with LGEO resulted in a $39.55 \pm 3.94\%$ mass increase in the first 3 days. No significant mass variation was observed in the remaining 45 days (Table S2 and Fig. S3). Additionally, the experiment revealed that pure citral interacted slightly more with the

polymeric matrix, resulting in a $58.31 \pm 8.46\%$ mass increase in the first 3 days. The increased polymer swelling caused by citral may be attributed to its role as the only compound that interacts with the PLA. However, even though citral slightly increased the PLA entumescence compared to LGEO, no mass variation was detected during the next 45 days. The results show that PLA is insoluble in neat citral, making it suitable for PLA encapsulation and permitting the controlled release of the encapsulated bioactive core.

Optimization of the Bioactive Core to the Polymer Wall Ratio, and Scaling-up of the Nanoencapsulation

The systematic optimization of the bioactive core-to-polymer ratio was a critical prerequisite for the proposed scale-up step, as it directly determines the amount of active compound delivered per unit volume of colloidal suspension without requiring additional concentration steps. As shown in Table 1, progressively increasing the volume of citral or LGEO from 100 to 500 μ L, while maintaining constant amounts of PLA (100 mg), Span 60 (77 mg), Tween 80 (77 mg), and phase volumes, did not compromise encapsulation efficiency (EE), which remained consistently high across all tested conditions (79.4–91.6% for citral and 90.0–94.1% for LGEO). EE tends to decrease with higher core loading in many polymeric systems due to the saturation of the polymer matrix and the displacement of the active compound toward the aqueous phase (Weisany et al., 2022). The stability of EE across the investigated loading range is likely due to the high affinity between the lipophilic bioactive core and the hydrophobic PLA matrix, combined with the surfactant system that stabilizes the oil-polymer interface during nanoprecipitation.

The increase in loading capacity was accompanied by an expected and moderate increase in the hydrodynamic mean diameter. Values ranged from approximately 140 nm (100 μ L core) to 220–224 nm (500 μ L core) for

Table 1 Formulations of PLA nanocapsules containing citral or LGEO as active core and the results obtained from the responses: *EE* encapsulation efficiency, *LC* loading capacity, *MD* hydrodynamic mean diameter, *ZP* zeta potential, and *PDI* polydispersity index

Formulation	Active compound (μ L)	EE (%)	LC (%)	MD (nm)	ZP (mV)	PDI
Citral-NC-1	100	89.09 ± 1.24^{ab}	44.37 ± 2.86^d	172 ± 18^c	-31.4 ± 0.9	0.149 ± 0.007
Citral-NC-2	200	87.96 ± 1.76^b	60.87 ± 3.07^c	195 ± 14^b	-27.7 ± 1.4	0.166 ± 0.004
Citral-NC-3	300	79.39 ± 0.79^c	65.36 ± 5.21^b	215 ± 10^a	-28.8 ± 0.7	0.156 ± 0.002
Citral-NC-4	400	91.63 ± 2.93^a	77.21 ± 1.59^a	219 ± 16^a	-26.4 ± 1.1	0.139 ± 0.008
Citral-NC-5	500	89.37 ± 3.31^{ab}	80.12 ± 1.85^a	220 ± 15^a	-28.5 ± 1.4	0.139 ± 0.002
LGEO-NC-1	100	91.22 ± 5.88^A	45.91 ± 2.50^D	177 ± 17^C	-31.1 ± 0.4	0.190 ± 0.003
LGEO-NC-2	200	89.99 ± 1.84^{AB}	61.96 ± 2.50^C	195 ± 10^B	-27.8 ± 0.9	0.148 ± 0.001
LGEO-NC-3	300	81.06 ± 0.71^B	66.30 ± 4.68^B	214 ± 9^A	-27.9 ± 1.5	0.178 ± 0.005
LGEO-NC-4	400	94.11 ± 0.78^A	78.02 ± 1.05^A	216 ± 13^A	-25.4 ± 0.9	0.136 ± 0.010
LGEO-NC-5	500	93.60 ± 1.11^{AB}	81.46 ± 1.01^A	224 ± 15^A	-26.3 ± 1.7	0.148 ± 0.009

both formulations. This increase in size can be attributed to the larger oily core volume available per PLA chain. Consequently, larger nanocapsules form as more oil is accommodated within each polymeric shell during the interfacial deposition (Fessi et al., 1989). Notably, even at the highest core loading (LGEO-NC-5 and Citral-NC-5), the mean diameters remained within the lower than 300 nm, considered favorable for colloidal stability and, potentially, for adhesion and retention at the fruit surface. The polydispersity index (<0.2) and the negative zeta potential values (below -25 mV) across all formulations further confirm the physicochemical homogeneity and electrostatic stability of the resulting colloidal suspensions. Zeta potential is a key indicator of colloidal stability, as it reflects the electrostatic repulsion between particles (Öztürk et al., 2024). Negative values in this range indicate that the nanocapsules are stabilized by repulsive electrostatic forces, which prevent aggregation and coalescence, thereby supporting a consistent and predictable shelf life.

From an economic standpoint, the LC values achieved in the optimized Citral-NC-5 and LGEO-NC-5 formulations are favorable, indicating that approximately 80% of the nanocapsule dry content corresponds to the encapsulated bioactive oil, with less than 20% attributable to the PLA wall material. Both citral and LGEO represent viable active cores depending on the application context: citral offers a standardized and well-characterized bioactive profile, while LGEO provides a naturally occurring product produced by direct steam distillation. In either case, the high LC of the optimized Citral-NC-5 and LGEO-NC-5 formulations ensures that the active oil constitutes the predominant fraction of the final product by mass, a key parameter for assessing the cost-effectiveness of the polymer contribution at an industrial scale.

The results are expressed as the mean of triplicates. According to the Tukey test ($p \leq 0.05$), values with the same letter after them do not differ.

After determining that Citral-NC-5 and LGEO-NC-5 offered the most favorable combination of loading capacity, particle size, and stability, they were selected for batch

scale-up. The scale-up process was performed at a 40-fold increase over the optimized laboratory scale, resulting in a production of 2 L of nanocapsule suspension (Fig. 2). A critical aspect of this process was the management of the acetone removal step, which was carried out under slightly reduced pressure (800 mbar) at 40 °C using an external water bath. This controlled evaporation setup was designed to prevent nanocapsule degradation under aggressive solvent-removal conditions, which can cause particle coalescence, size increase, and loss of encapsulation efficiency (Ephrem et al., 2014). Additionally, the controlled evaporation setup enables organic solvent recovery for future reuse, aligning with sustainable manufacturing principles. Comparative studies on rosemary essential oil nanocapsules produced by nanoprecipitation at small and large scales have shown that properties such as particle diameter, PDI, and zeta potential are well preserved across scales (Ephrem et al., 2014), underscoring the inherent scalability of this method. The results presented in the scale-up method were closely comparable to the optimized laboratory scale, with Citral-NC-5 and LGEO-NC-5 maintaining EE values of 84% and 87%, mean hydrodynamic diameters of 217 and 221 nm and zeta potential of -28.7 mV and -27.4 mV, respectively (Table 1).

The FEG-SEM micrographs confirmed the spherical morphology of the nanocapsules at the scale-up level (Fig. 3), with mean diameters of 124 ± 31 nm for Citral-NC-5 and 171 ± 25 nm for LGEO-NC-5 as measured from image analysis (Fig. S4).

Successful scale-up of both formulations while preserving their physicochemical characteristics is a significant step toward practical, commercial-scale food preservation applications. The nanoprecipitation technique has demonstrated good reproducibility when transitioning from the laboratory to a larger scale. The use of an automated dosing pump with a constant injection rate of 0.5 mL min^{-1} was essential in controlling the hydrodynamic conditions at the organic-aqueous interface. This parameter is known to critically influence particle size and distribution during solvent displacement (Bilati et al., 2005). Injecting the organic phase

Fig. 2 Laboratory scale-up batch process for PLA-encapsulation of citral or LGEO. **A** Organic phase addition to the aqueous phase. **B** Homogenization. **C** Organic solvent evaporation and recovery. **D** Final 2-L non-encapsulated colloidal solution

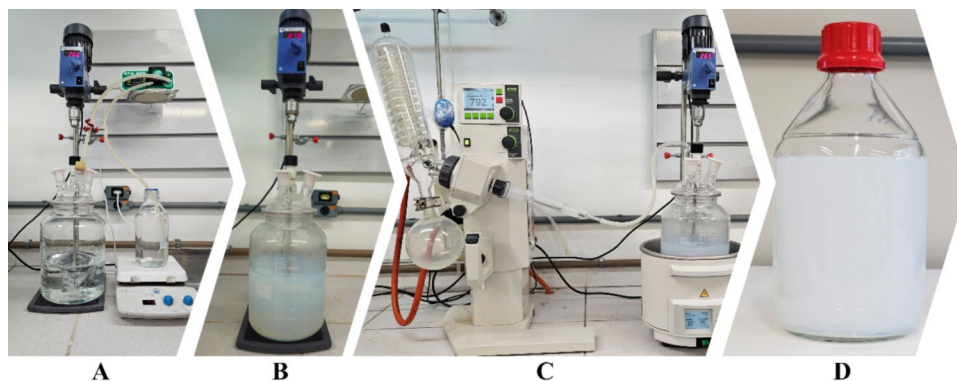
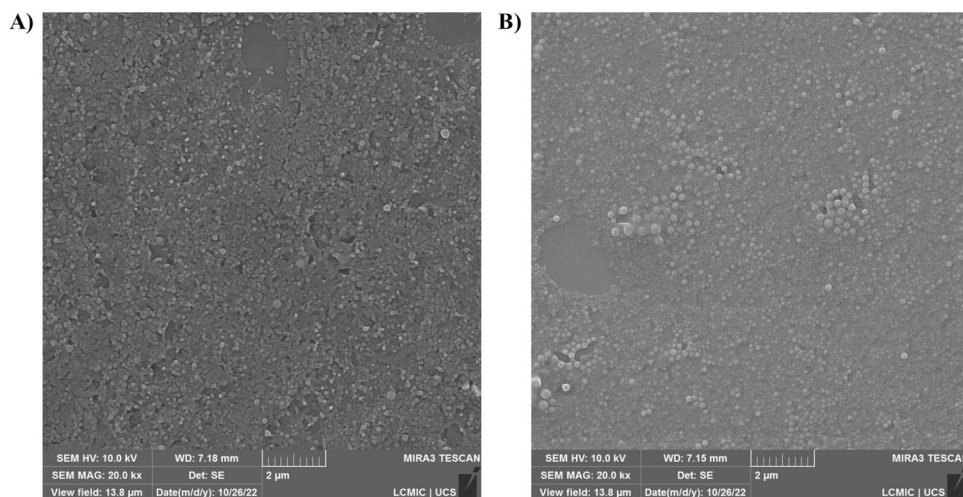


Fig. 3 Micrographs obtained by field emission gun scanning electron microscopy (FEG–SEM) of the scaled-up **A** Citral–NC–5 and **B** LGEO–NC–5



into the aqueous phase, rather than simply pouring one phase over the other, has been shown to significantly reduce particle size by promoting more controlled interfacial turbulence (Bilati et al., 2005). This is consistent with the narrow size distributions observed in this study. Additionally, recovering acetone under reduced pressure not only prevents loss of encapsulated volatile compounds but also aligns with sustainable manufacturing principles by enabling solvent reuse. This is a relevant consideration when envisioning large-scale postharvest applications.

Another factor for the practical application of these formulations is their long-term storage stability. In this context the storage stability of Citral–NC–5 and LGEO–NC–5 was evaluated over a 90-day period under refrigerated conditions (4 °C). After 90 days, both formulations maintained their physicochemical characteristics, with mean hydrodynamic diameters of 226 nm for Citral–NC–5 and 219 nm for LGEO–NC–5, remaining consistent with the values obtained immediately after production which were 217 nm and 221 nm, respectively. Similarly, the registered zeta potential values were close to the results obtained after formulation, with values of –27.9 mV for Citral–NC–5 and –27.6 mV for LGEO–NC–5. In addition, the EE was also measured showing minimal variation, with values of 85% and 83% for Citral–NC–5 and LGEO–NC–5, respectively. These results indicate that the nanocapsules exhibit good colloidal stability and retention of the bioactive core under refrigerated storage, which is particularly relevant for postharvest applications involving cold-chain conditions.

It is worth mentioning that all nanocapsule suspension's components are either biodegradable, food-grade, or GRAS-classified, and thus, the presence of residual material on fruit surface does not raise food safety concerns under the proposed application conditions. PLA undergoes hydrolytic degradation to lactic acid, a naturally occurring metabolite. The surfactants sorbitan monostearate (Span 60) and

polysorbate 80 (Tween 80) are approved food additives, and citral and LGEO are widely used as food flavoring agents (Jackson-Davis et al., 2023; Wi et al., 2025). Therefore, incidental ingestion of residual nanocapsule material does not raise food safety concerns.

Conidia Germination Assay for *B. cinerea*

Conidial germination assays were conducted to evaluate and compare the antifungal efficacy of Citral–NC–5 with non-encapsulated citral, as well as the LGEO–NC–5 and non-encapsulated LGEO. This assay provides important insights into the ability of these treatments to interfere with the early developmental stage of *B. cinerea*, which is critical for fungal infection and disease progression.

Table 2 In vitro antifungal activity of the encapsulated and non-encapsulated citral and LGEO against *B. cinerea* after 24 h

Treatment concentration % (v/v) ^a	Conidia germination scale ^b			
	Citral	Citral–NC–5	LGEO	LGEO–NC–5
0.4	III	III	III	III
0.2	III	III	III	III
0.1	III	III	III	III
0.05	III	III	III	III
0.025	II	II	I	I
0.0125	I	I	I	I
0.00625	I	I	I	I
Control	I	I	I	I

^aThe final concentrations of the encapsulated citral and LGEO or citral were prepared from the scale-up formulations of Citral–NC–5 and LGEO–NC–5 with initial concentrations of 0.84% (v/v) and 0.87% (v/v), respectively. ^b(I) conidia with hyphal growth, (II) conidia with germ tube emergence, and (III) conidia with no observable hyphal emergence

As shown in Table 2, at concentrations higher than 0.05% (v/v), citral, Citral-NC-5, LGEO, and LGEO-NC-5 completely inhibited conidial germination (scale III). At a concentration of 0.025% (v/v), both citral and Citral-NC-5 were able to partially reduce conidial germination, with the presence of conidia exhibiting germ tube emergence (scale II). In contrast, at this same concentration, LGEO and LGEO-NC-5 were no longer effective in controlling the conidial germination of *B. cinerea*. At concentrations below 0.0125% (v/v), none of the evaluated treatments was effective in inhibiting conidial germination.

Flow Cytometry

Flow cytometry was employed to investigate the effect of encapsulated and non-encapsulated citral and LGEO on the physiological state of *B. cinerea* conidia, focusing on plasma membrane integrity and intracellular esterase activity.

Based on the in vitro germination test, the concentrations of encapsulated and non-encapsulated citral and LGEO at 0.05% (v/v) and 0.10% (v/v) were selected. In the untreated control (Fig. 4A), 66.23% of the conidia population exhibited esterase activity (quadrants 2 and 3), confirming active metabolic status, while 93.4% of the population showed no PI uptake (quadrant 4), indicating intact plasma membranes. It should be noted that the comparatively high proportion of conidia in quadrant 4, exhibiting low CFDA-AM fluorescence, is consistent with previously reported behavior of *B. cinerea* conidia, in which the thickness and hydrophobicity of the conidial wall can limit dye penetration, reducing

detectable esterase activity relative to actively germinating cells or hyphae (Romero Bernal et al., 2019).

Following treatment with encapsulated or non-encapsulated citral and LGEO at 0.05% and 0.10% (v/v), both concentrations induced a measurable shift in the conidia population, characterized by a simultaneous increase in PI uptake and redistribution of CFDA-AM fluorescence toward quadrants 2 and 3 (Fig. 4B to I). These patterns are indicative of two complementary processes: partial disruption of plasma membrane integrity, evidenced by the increased permeability to PI and enhanced intracellular esterase activity (Scariot et al., 2020).

The mechanism by which citral and LGEO compromise fungal membrane integrity is consistent with the lipophilic nature of monoterpene aldehydes. Citral, the predominant bioactive component of both formulations, can partition into the phospholipid bilayer, disrupting membrane fluidity and integrity through interactions with membrane lipids and proteins (Pedroso et al., 2024; Scariot et al., 2020). Additionally, evidence from reported studies suggests that citral interferes with ergosterol biosynthesis, which is a critical sterol for maintaining the structural integrity and membrane fluidity of fungi (OuYang et al., 2016; Sousa et al., 2016). Depletion of ergosterol compromises the organization of the lipid bilayer, increasing membrane permeability, resulting in the leakage of intracellular components and loss of cellular homeostasis (Leite et al., 2014). For instance, geranyl increased the PI fluorescence for *Trichophyton rubrum* conidia within 24 h of treatment (Zheng et al., 2021). It also led to a significant reduction in ergosterol content, corroborating the membrane-damaging capacity observed in

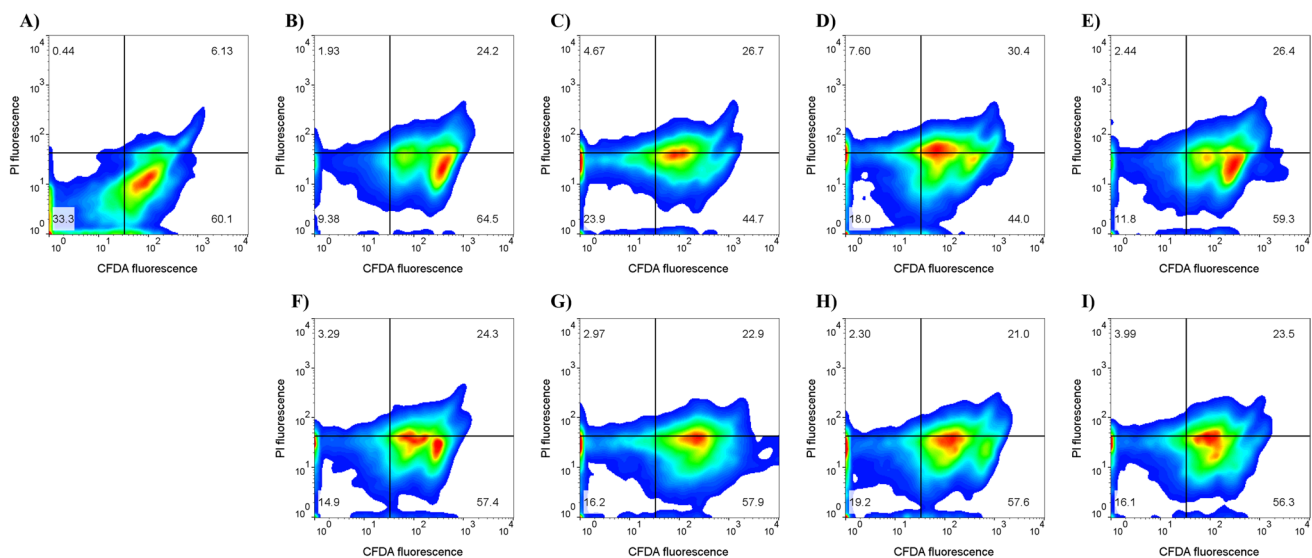


Fig. 4 Effect of encapsulated and non-encapsulated citral and LGEO on *B. cinerea* conidia on esterase activity and membrane integrity. **A** Control; **B** Citral 0.05% (v/v); **C** Citral 0.10% (v/v); **D** LGEO 0.05%

(v/v); **E** LGEO 0.10% (v/v); **F** Citral-NC-5 0.05% (v/v); **G** Citral-NC-5 0.10% (v/v); **H** LGEO-NC-5 0.05% (v/v); **I** LGEO-NC-5 0.10% (v/v)

this study. Similarly, the combination of citral and cinnamaldehyde against *Penicillium expansum* induced a significant decrease in ergosterol content, along with detectable changes in membrane phospholipid and fatty acid composition, further supporting a multi-target membrane disruption mechanism (Wang et al., 2018).

Although no fully PI fluorescence was detected for conidia population under the tested conditions, the observed redistribution patterns collectively indicate that citral and LGEO, in both encapsulated and free forms, place *B. cinerea* conidia under significant membrane stress, a state that, while not immediately lethal at these concentrations within the 6-h assay window, is consistent with inhibited germination. This interpretation aligns directly with the in vitro germination data (Conidia Germination Assay for *B. cinerea*), in which complete inhibition of conidial germination was observed at concentrations $\geq 0.05\%$ (v/v).

Evaluation of Gray Mold Control in Grapes

In this study, grapes were chosen as an in vivo model to validate the antifungal activity of the nanoencapsulated formulations, given the worldwide impact of gray mold on grapes. Gray mold causes a significant decline in the quantity and quality of mature berries (Rahman et al., 2024). In addition, necrosis progression caused by the fungus can easily be measured due to the visible symptoms on the berry's surface. Based on the in vitro findings, the encapsulated and non-encapsulated citral and LGEO were chosen at concentrations of 0.05% (v/v) and 0.10% (v/v), and lesion diameters were evaluated at 96 h, 120 h, and 168 h.

In the comparative study involving the LGEO, it was demonstrated that the untreated grapes developed extensive necrotic decay, with lesion diameters increasing progressively during the evaluation period. Grapes treated with LGEO at concentrations of 0.05% and 0.10% showed no significant efficacy in preventing necrotic decay compared to the negative control group, as shown in the bar chart in Fig. 5A. In contrast, LGEO-NC-5 treatments inhibited the expansion of the lesions and disease severity. Additionally, a dose-dependent effect was observed. The LGEO-NC-5 at a concentration of 0.10% (v/v) exhibited the most significant reduction in fungal progression, with a 63% reduction in lesion diameters after 168 h when compared to the negative control, as shown in Fig. 5A.

Moreover, no necrotic decay was observed in the uninoculated control. When the lesion diameters of the same treated group were compared at three different time points (Fig. 5B), it was found that LGEO-NC-5 at a concentration of 0.10% (v/v) slowed the decay rate of the berries. There was no statistically significant progression of the infection over the evaluated period. The visual assessment of the grape berries depicted in Fig. 5C corroborates the quantitative data. The

control and non-encapsulated treatment groups had berries exhibiting severe symptoms, including widespread browning, mycelial growth, and tissue collapse. In our previous studies, we also observed that lesion sizes were similar to or larger than those in the negative control group when non-encapsulated essential oil was used (Antonioli et al., 2020; Molon et al., 2026). In contrast, grapes treated with LGEO-NC-5 displayed only minor superficial lesions, and overall tissue integrity was preserved, particularly with the treatment at a concentration of 0.10% (v/v).

The in vivo evaluation using citral and Citral-NC-5 yielded similar results to those observed in the experiments with LGEO and LGEO-NC-5. However, during the first 96 h, the grapes treated with non-encapsulated citral showed larger lesions than those in the negative control group. This pattern followed through the subsequent measurements, as shown in Fig. 6A. The pronounced phytotoxicity, compared to LGEO, can result from the fact that citral consists only of geranial and neral. Although citral has well-known antimicrobial activity, it can exhibit phytotoxicity, causing adverse effects on fruits or plants (Abd-Elgawad et al., 2021).

On the other hand, grapes treated with encapsulated citral exhibited a significant reduction in disease lesions. At the first measurement, after 96 h, grapes treated with a colloidal suspension of Citral-NC-5 at a concentration of 0.05% (v/v) had lesions that were 27% less severe than those in the negative control group. Greater gray mold control was observed when the grapes were treated with Citral-NC-5 at 0.10%. The average diameter of the lesions was 1.87 ± 1.0 mm, which is 72% smaller than the diameter of the lesions in the control group, which was 6.75 ± 2.3 mm. Moreover, when comparing the mean diameter of the lesions between treatments with non-encapsulated and encapsulated citral at a concentration of 0.10%, the effect of encapsulating the bioactive compound was evident. In addition to inhibiting the increase in the lesion, it also inhibited the formation of mycelium on the surface. Similar to the grapes treated with LGEO-NC-5 at 0.10% (v/v), those treated with Citral-NC-5 at the same concentration showed a slower progression of infection over the evaluation period.

The superior performance of the encapsulated formulations compared to non-encapsulated citral and LGEO suggests that nanoencapsulation plays a critical role in enhancing antifungal efficacy. This effect can be attributed to the sustained release of the active compounds along with the protective effect supported by the nanocapsules, thereby prolonging their bioavailability on the fruit surface (Reyes-Jurado et al., 2020). In addition, incorporating hydrophobic compounds into nanocarriers improves their dispersion in aqueous environments, facilitating more effective contact with fungal structures (Nazzaro et al., 2017). Furthermore, previous studies have demonstrated that nanoencapsulation of essential oils can reduce phytotoxic effects by modulating

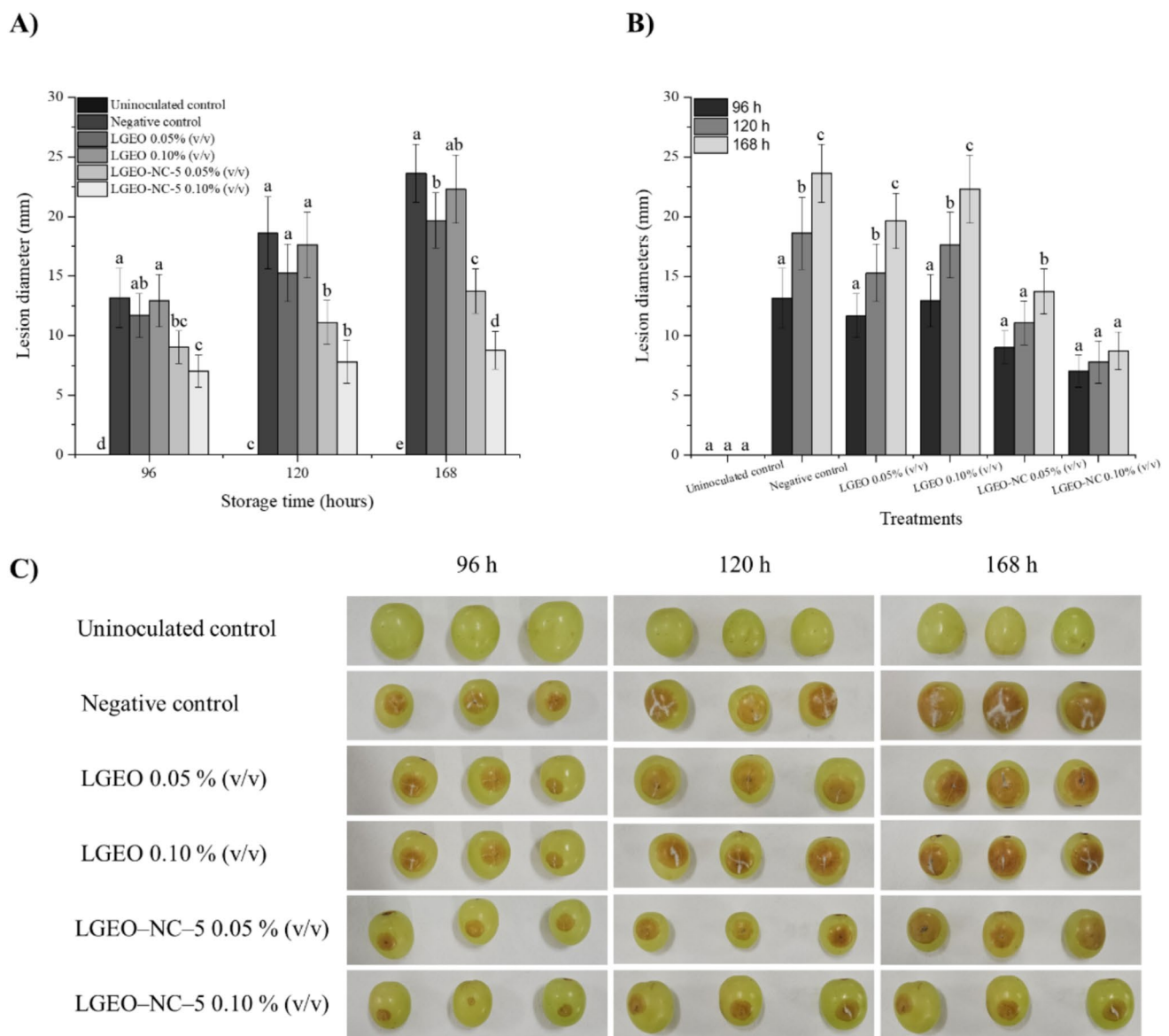


Fig. 5 In vivo preventive postharvest treatment against *B. cinerea* in grapes treated with non-encapsulated and encapsulated LGEO. **A** Lesion diameter of grape berries inoculated with *B. cinerea* conidia measured at three storage times for the six different treatments. For each storage time, the same letters indicate the absence of statistical differences within the treatments according to Tukey's test ($p \leq 0.05$). **B** Bar chart showing the progression of infection in the same treat-

ment group over the storage times. For each treatment, the same letters indicate the absence of statistical difference within the treatments according to Tukey's test ($p \leq 0.05$). **C** Images from three grapes, which represent the average treatment group after 168 h of incubation. The measured data are detailed in Table S3, and the images of all barriers are shown in Fig. S6

the release of active compounds (Antonioli et al., 2020; Molon et al., 2026), which is consistent with the reduced phytotoxicity observed for the encapsulated formulations in the present study.

After 168 h, the grapes were sliced to assess the internal integrity (Figs. 7 and 8). Noticeable differences in coloration were observed inside the grape berries. Berries treated

with non-encapsulated LGEO or citral showed internal decay that was similar to that seen in the berries of the negative control group. In contrast, the internal structure of the grape berries treated with encapsulated LGEO or citral exhibited no signs of fungal deterioration. The internal part of these berries resembled the uninoculated control, regardless of the evaluated concentration.

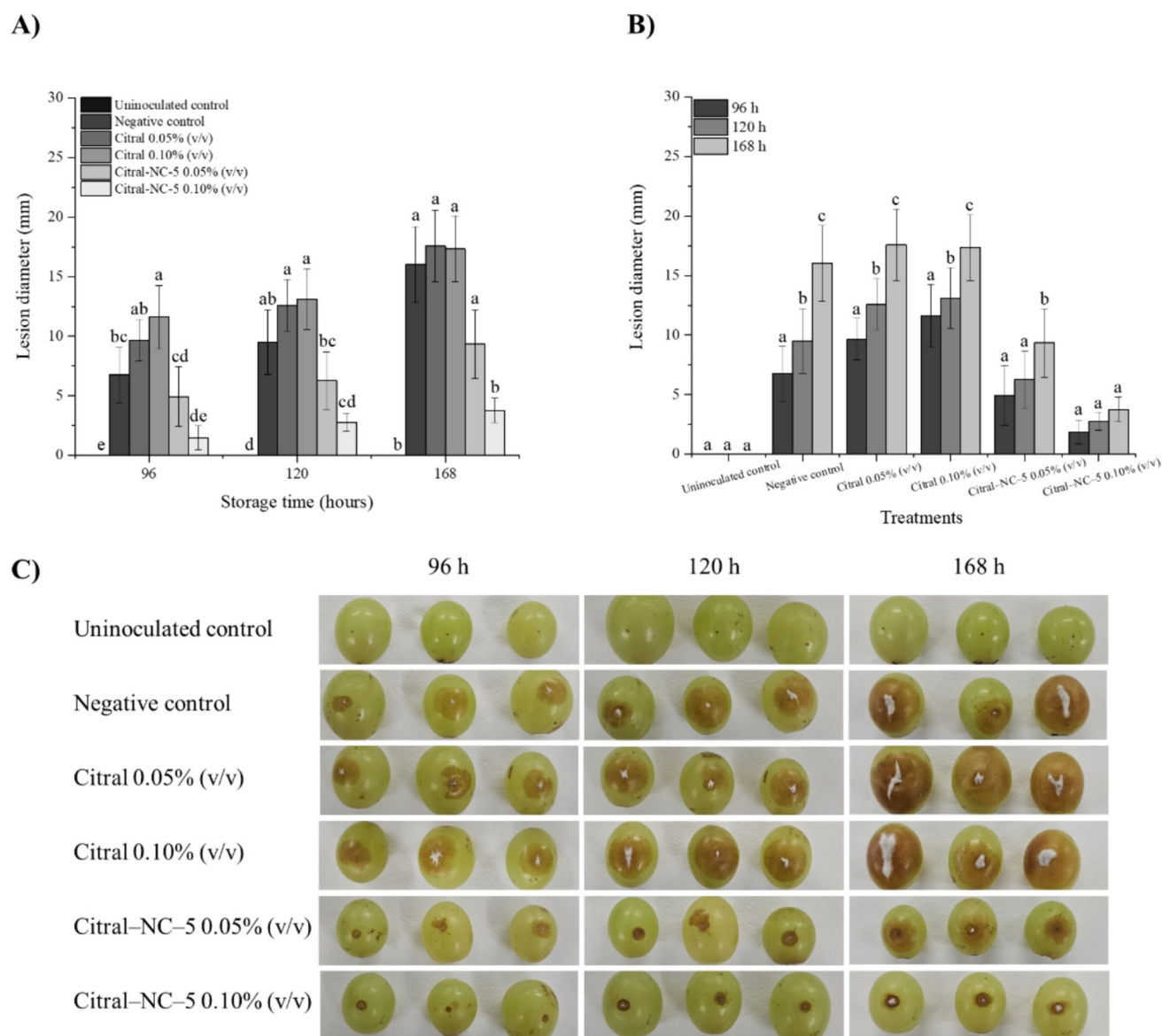


Fig. 6 In vivo preventive postharvest treatment against *B. cinerea* in grapes treated with non-encapsulated and encapsulated citral. **A** Lesion diameter of grape berries inoculated with *B. cinerea* conidia measured at three storage times for the six different treatments. For each storage time, the same letters indicate the absence of statistical differences within the treatments according to Tukey's test ($p \leq 0.05$). **B** Bar chart showing the progression of infection in the same treat-

ment group over the storage times. For each treatment, the same letters indicate the absence of statistical difference within the treatments according to Tukey's test ($p \leq 0.05$). **C** Images from three grapes, which represent the average treatment group after 168 h of incubation. The measured data are detailed in Table S4, and the images of all barriers are shown in Fig. S7

Conclusions

In this study, we successfully scaled up the production of PLA-nanoencapsulated citral and lemongrass essential oil using an accessible and scalable nanoprecipitation approach. The resulting nanostructures proved highly effective in preventing the conidia germination of *B. cinerea*, a devastating necrotrophic pathogen responsible for significant postharvest losses. A key contribution of

this work lies in the comparative analysis between free and encapsulated bioactive compounds during in vivo applications. Our findings demonstrate that while free essential oils possess antifungal potential, their direct application is limited by high phytotoxicity, as evidenced by the necrotic lesions observed in treated grape berries. Nanoencapsulation successfully overcame this limitation, providing a controlled-release mechanism that preserves antifungal activity while protecting host tissue. Therefore,

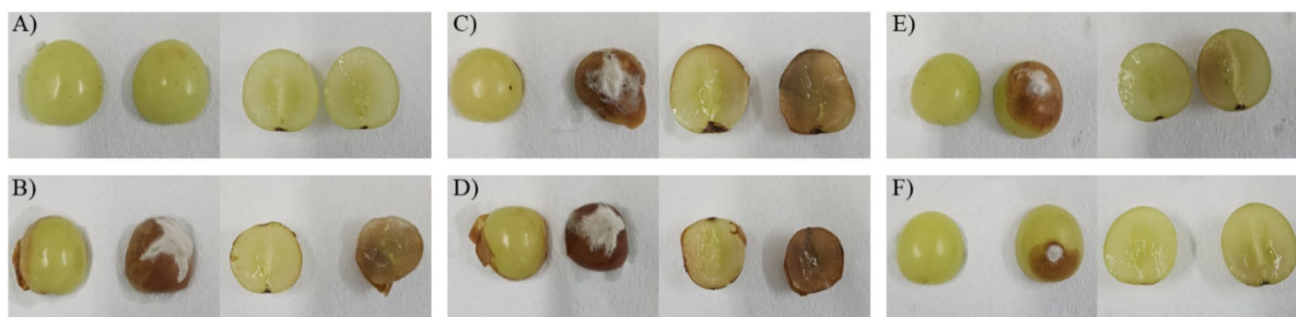


Fig. 7 Sliced grapes selected from each in vivo treatment group after 168 h. Each image shows the same berry: the skin on the left and the inside on the right. **A** Uninoculated control; **B** negative control;

C LGEO 0.05% (v/v); **D** LGEO 0.10% (v/v); **E** LGEO-NC-5 0.05% (v/v), and **F** LGEO-NC-5 0.10% (v/v)

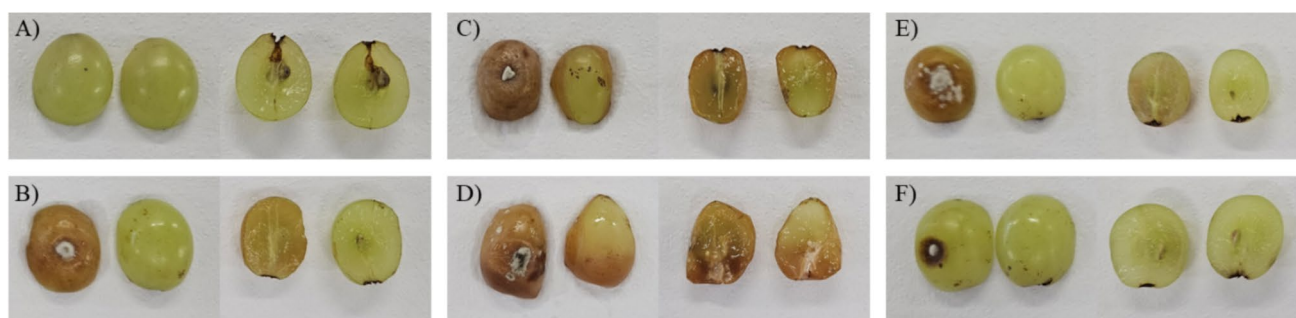


Fig. 8 Sliced grapes selected from each in vivo treatment group after 168 h. Each image shows the same berry: the skin on the left and the inside on the right. **A** Uninoculated control. **B** Negative control. **C**

citral 0.05% (v/v). **D** citral 0.10% (v/v). **E** Citral-NC-5 0.05% (v/v). **F** Citral-NC-5 0.10% (v/v)

the development of these biodegradable polymer-based nanocarriers represents a significant advancement in green nanotechnology for the food industry.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11947-026-04438-3>.

Acknowledgements We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the DAI CNPq PhD scholarship received by V.B.M., the PIBIT-CNPq undergraduate scholarship received by M.P.O., and the research fellowship received by T.B. FAPERGS is also acknowledged for the PROBIC undergraduate scholarship received by B.S.V.

Author Contribution V.B.M.: conceptualization, methodology, investigation, validation, formal analysis, writing – original draft; Writing – review & editing. M.P.O.: investigation; B.S.V.: investigation. F.J.E.: methodology, investigation; A.P.L.D.: resources; T.B.: conceptualization, methodology, resources, funding acquisition, project administration, supervision, writing-review and editing. All authors have read and agreed to the published version of the manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). This work was financially supported by Fundação de

Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, grant: PqG 21/2551-0002113-6) and Launer Química Indústria e Comércio Ltda. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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