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# Production of a Starch-Based Polymeric Coating with Incorporation of Bioactive Principles from Chemical Synthesis to Extend the Shelf Life of Cavendish Banana

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# Production of a Starch-Based Polymeric Coating with Incorporation of Bioactive Principles from Chemical Synthesis to Extend the Shelf Life of Cavendish Banana

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**Abstract:** Crown rot is a severe postharvest disease affecting tropical and subtropical fruits like mango, avocado, papaya, and banana. It is caused by fungal pathogens that penetrate the fruit, reducing its pulp and leading to premature ripening. Systemic fungicides have been used to control these fungi, typically applied to seeds, leaves, or fruits to prevent disease spread. However, traditional fungicides can pose toxicity risks to the environment and human health. Essential oils are chemical substances that can be found in plants and have antifungal capacity. Essential oils are being investigated as an alternative to traditional fungicides since they are less toxic to the environment and human health; however, they are more expensive and less efficient than traditional fungicides. Accordingly, chemically synthesizing the chemical compounds that are the active antifungal agent inside essential oils can be an ecological and effective approach to produce a new generation of antifungals. In this study, modified starch was investigated as a carrier for thymol (active antifungal agent in thyme oil) incorporation using four distinct methods. Emulsions of starch and thymol were prepared and spray dried to obtain a soluble powder that was used to produce coatings. The most effective method for thymol incorporation yields a retention of approximately 40% according to gas chromatography analysis. In-vitro results indicated that thymol incorporated into the matrix exhibited antifungal effects against key fungi responsible for crown rot disease in Cavendish bananas at concentrations greater than 6% w/w relative to the coating matrix.

**Keywords:** Crown Rot, Antifungal Coating, Banana Postharvest Treatment, Thymol

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# Introduction

In terms of export value, banana is the most important fruit globally. In 2022, global banana exportations reached 19.2 million tons [1]. Today, this fruit boasts a significant genetic diversity base that still exists due to natural crossbreeding [2]. One single variety, Cavendish, dominates 40% of global banana crops, representing nearly all large-scale export trade and a significant portion of local trade [3]. The Cavendish is a seedless sweet banana variety classified within the AAA group. The food security of less developed or low-income countries depends heavily on bananas due to their high consumption and potential [4]. The Cavendish banana currently dominates the global market but faces ongoing challenges, particularly in preventing postharvest diseases like crown rot, a damaging affliction affecting a wide range of tropical and subtropical fruits [5].

Crown rot is considered the primary postharvest disease affecting bananas, causing losses of around 20-25%. This critical disease is caused by fungi, including *Colletotrichum musae*, *Lasiodiplodia theobromae*, and *Fusarium sp.* [6,7]. Systemic fungicides have traditionally been used to control these fungi, but these products can be harmful to the environment and human health [8]. The European Union has restricted maximum residue limits (MRLs) for several fungicides, increasing the need to explore or develop safer alternatives [9]. Essential oils are a promising alternative due to their antifungal properties and lower toxicity. Studies have shown that essential oils from plants like thyme and lemongrass are effective against a wide range of pathogens [10-12].

In this context, to enhance control of crown rot in Cavendish bananas, the Integrated Design Group for Processes and Products (GDPP) at Universidad de los Andes has developed a natural coating based on modified cassava starch. This coating has been evaluated on Cavendish bananas incorporating thyme oil as an antifungal agent, demonstrating promising results as a postharvest treatment for maintaining banana quality and extending shelf life [13]. Therefore, it is possible to harness the primary bioactive compounds of essential oils, such as thymol and citral, which can be chemically synthesized to address their limitations, such as cost and efficacy [14,15]. This research investigates the effectiveness of this polymeric coating with thymol (from chemical synthesis) incorporation to improve the scalability of the coating as an alternative postharvest treatment for Cavendish bananas.

# Materials and Methods

## Materials

Native cassava starch, glycerol, citric acid, and other raw materials for coating matrix elaboration were supplied by Cimpa®. Sunflower oil was purchased from a commercial store and thymol crystals from Sigma Aldrich® were used. The *Fusarium sp.* and *Colletotrichum sp.* strains were provided by the Banana Research Center (Cenibanano), along with Cavendish bananas (*Musa AAA*).

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## Coating Preparation and Thymol Incorporation

The preparation of the coating matrix was carried out as reported by Vaca et al. [16]. This process, shown in Figure 1, began with the mixing of citric acid, glycerol, and cassava starch, and other raw materials, followed by modification and drying through spray drying. Detailed process information can be found in patent application WO2023026073. This coating matrix will hereafter be referred to as SM.

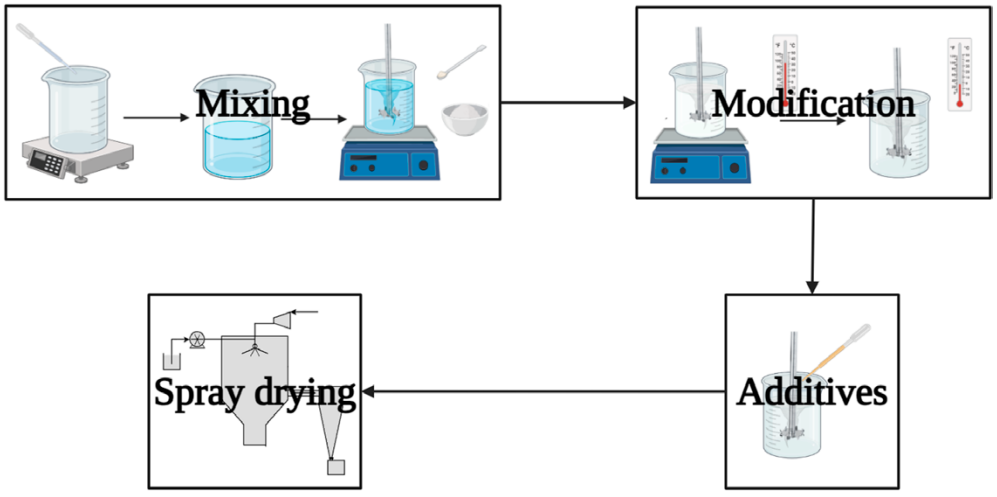


**Figure 1.** Coating matrix preparation method. Based on what is reported in the patent application WO2023026073. Created in Biorender.com

Once the SM coating matrix was obtained, thymol was incorporated by impregnating the obtained powder with a thymol solution in sunflower oil at a concentration of 10% w/v of thymol in oil. The solution of thymol in oil was added to SM powder through a dropwise method in a relation of 10% v/w relative to SM, to produce a 1% w/w thymol concentration in coating, then mixed and sealed hermetically in a vial, which was left to stabilize for 4 days. This formulation will hereafter be referred to as SM-TH.

## Characterization of Thymol Incorporation

### Thermogravimetric Analysis:

A thermogravimetric analysis was performed using a TA Instruments Q600® (New Castle, DE, USA). The method involved a ramp of 10°C/min up to 600°C in a nitrogen atmosphere with a flow rate of 10 mL/min. Using this methodology, the SM and SM-TH formulations were compared.

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## Gas Chromatography Analysis:

The presence of thymol in the coating sample was verified using gas chromatography (GC). An ethanol extraction was performed on the coating with thymol sample (SM-TH) and the non-thymol coating or coating matrix sample (SM) during 24 h in a 1:10 coating-to-ethanol ratio. These extracts were then compared to a thymol standard solution in ethanol. A calibration curve was constructed to quantify the incorporated thymol using this method.

A methodology previously used by the research group was employed for the GC analysis. A Shimadzu MDGC-2010 gas chromatograph was utilized, configured as follows: an RTX-WAX column (100% polyethylene glycol) measuring 30 m × 0.25 mm × 0.5 µm was used. The oven temperature was initially set at 35°C for 2 minutes, followed by a heating rate of 5°C/min until reaching 150°C. Subsequently, the temperature was increased to 250°C at a rate of 10°C/min and held for 7 min to eliminate residues. The injector temperature was fixed at 250°C, utilizing the splitless injection mode, with 2 µL of the sample being injected. A flame ionization detector (FID) was used at 250°C. Helium was employed as the carrier gas at a flow rate of 1 mL/min.

## In-vitro evaluation:

Initially, *Fusarium sp.* and *Colletotrichum sp.* strains were activated and replicated to obtain mother cultures of each strain, received using the filter paper conservation method. Conidial suspensions of the pathogens were prepared from each mother culture, with a concentration of $1x10^3$ conidia per milliliter and a 1:1 ratio of conidia from each strain. For the in-vitro tests, 100 µL of the conidial suspension was inoculated, followed by the application of 400 µL of the coating formulations. The coatings were allowed to dry and then incubated at 27°C for 48 hours. This methodology is detailed in Figure 2. Starting from the formulation (SM-TH), the thymol concentration was increased until achieving a significant colonies inhibitory effect comparing to the conventionally used fungicide, Mertect®, containing thiabendazole as its active ingredient, which served as the positive control. The formulations used in the reported in vitro test are listed in Table 1. Each coating formulation was prepared by reconstituting 1.5 g of powder in 100 mL of sterile distilled water at room temperature.

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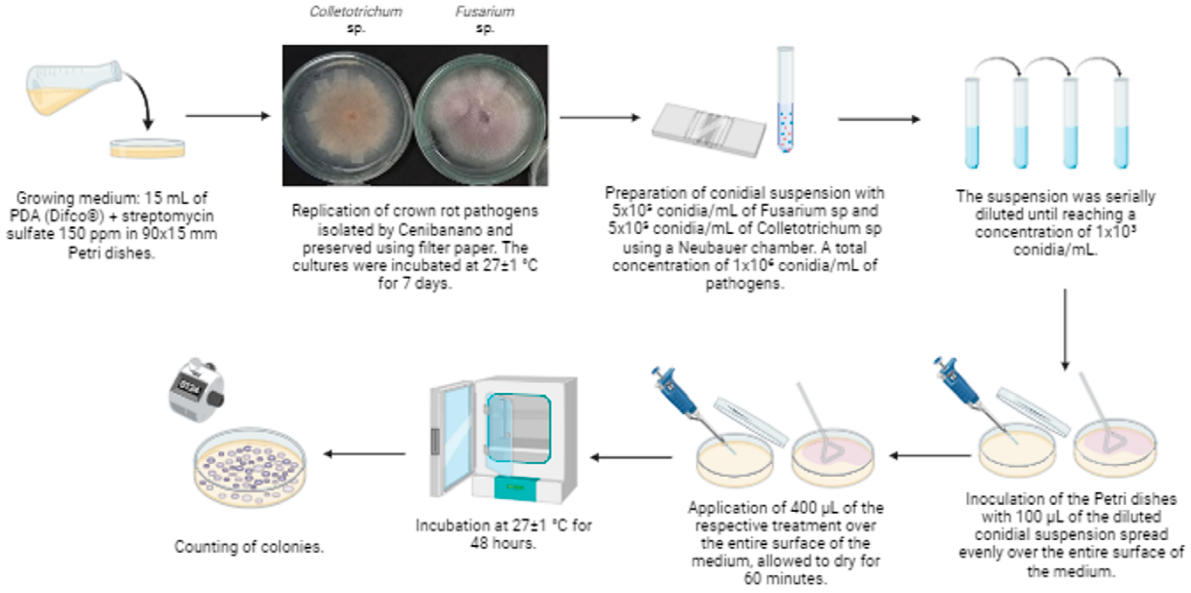


**Figure 2.** In-vitro evaluation methodology. Created in Biorender.com

**Table 1.** In-vitro coating formulations and control treatments.

| Formulations | Inoculation | Thiabendazole[1] | Starch Matrix | Sunflower oil | Thymol[2] |
|---|---|---|---|---|---|
| C- | X | - | - | - | - |
| C+ | X | 396 ppm | - | - | - |
| SM | X | - | X | - | - |
| SM-TH | X | - | X | X | 10 mg/g |
| SM-TH×6 | X | - | X | X | 30 mg/g |
| SM-TH×12 | X | - | X | X | 60 mg/g |

[1] Thiabendazole was applied in sterile deionized water solution at the concentration shown in the table.
[2] The concentrations shown for thymol are relative to the coating matrix.

# Results and Discussion

## Characterization of Thymol Incorporation

### Thermogravimetric Analysis:

Through the thermogram shown in Figure 3, it was observed that SM exhibits a significant drop around 50°C compared with SM-TH; typically, these drops at low temperatures are associated

with water loss, indicating that SM contains more water. Additionally, another weight loss occurs around 300°C; at this temperature, both formulations exhibit characteristic weight loss that is similar for both, associated with the degradation of the starch matrix, which is the main component of the coating. Another common drop for both occurs at 400°C, with SM-TH showing a higher loss, consistent with expectations due to the incorporation of oil as seen in Figure 3(a). Although the incorporation of oil in the coating through impregnation method is evident from TGA, the presence and loss of thymol are less distinct. Thymol typically exhibits characteristic weight loss around 140-160°C according to the planned methodology, yet no noticeable differences between SM and SM-TH are observed within this interval (see Figure 3[b]).

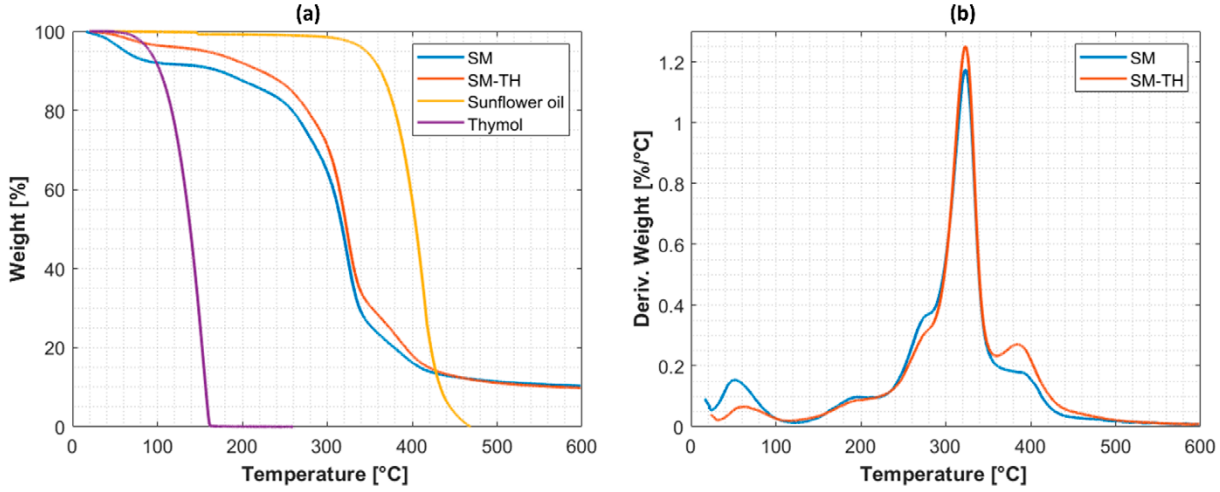


**Figure 3.** Thermograms of the coating matrix and the coating with thymol. (a) Weight percentage as a function of temperature (b) Derivative of weight as a function of temperature.

## Gas Chromatography Analysis:

In contrast to the TGA, the chromatograms of the ethanol solutions resulting from thymol and the extracts of the coatings respectively show a notable presence of thymol in SM-TH. The results are shown in Figure 4. In the chromatogram of the ethanol-thymol standard solution, the solvent (ethanol) elution is observed early on, around 4 min, and a second peak corresponding to thymol is seen at a retention time of approximately 24.5 minutes. Comparing SM and SM-TH, it is evident that SM-TH has a prominent thymol peak at the same retention time, whereas SM shows a very faint peak. This small peak in SM might be due to sample contamination during storage or residuals in the chromatograph column. Considering these observations, the presence of thymol in SM-TH is evident. After constructing a calibration curve with standards, it was determined that the average concentration for SM-TH is around 4±1 mg/g, equal to 0.4% w/w relative to SM. This result is the average of three measurements indicating that the incorporation capacity through impregnation method is near 40% of added thymol.

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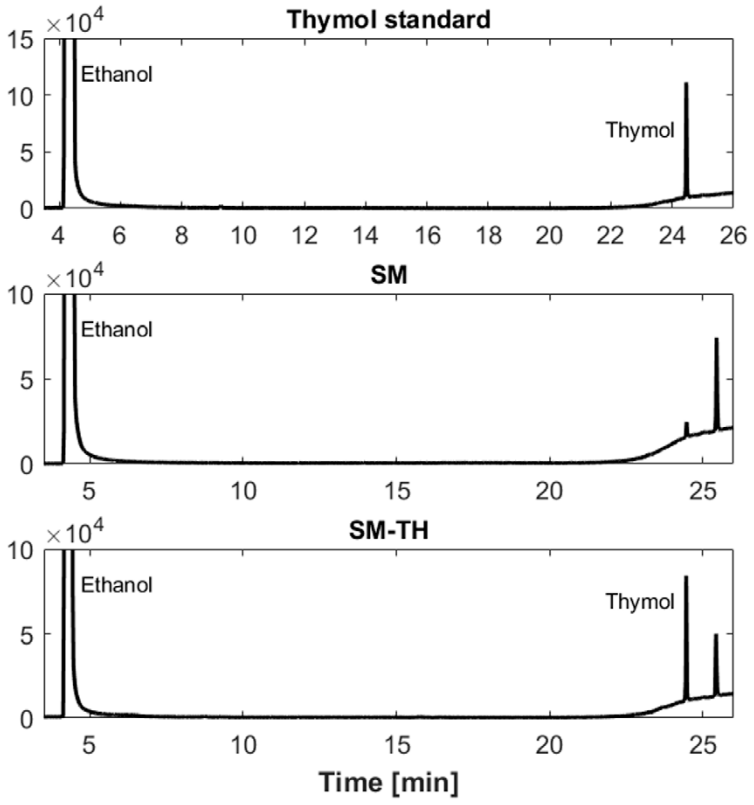


**Figure 4.** Chromatograms of thymol solution in ethanol and ethanol extracts of the coating matrix and coating with thymol incorporation.

## In-vitro evaluation:

The results of pathogen colony inhibition by the coatings are depicted in Figure 5. It is noted that SM shows similarity to the negative control, suggesting no significant effect solely from the coating matrix. A similar result was obtained with SM-TH, where despite containing 1% w/w thymol relative to the matrix (equivalent to 10 mg/g), no significant inhibitory effect is observed. However, when evaluating SM-TH at different concentrations, a notable inhibitory effect on the colonies is observed starting from a thymol concentration of 60 mg/g relative to the matrix (SM-TH×6). Increasing the concentration to 120 mg/g of the matrix (SM-TH×12) resulted in an inhibitory effect comparable to the positive control (C+).

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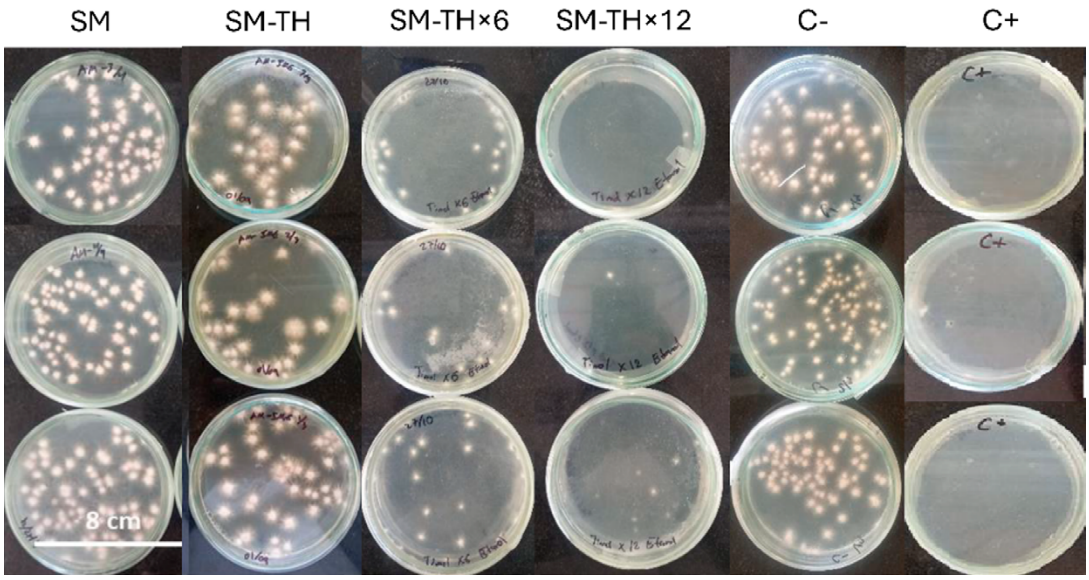


**Figure 5.** In-vitro pathogen colony inhibition test.

## Conclusions

In this study, the effectiveness of thymol incorporation into modified starch-based coatings for antifungal control was achieved. Thermographic analysis (TGA) reveals distinct thermal behaviors between formulations, with SM-TH demonstrating oil integration and less water content. Chromatographic analysis confirmed substantial thymol presence in SM-TH, demonstrating successful thymol incorporation in coating. In vitro testing against fungal pathogens showed that while SM alone exhibited minimal inhibitory effects, SM-TH displayed significant colony inhibition at higher thymol concentrations (more than 60 mg/g), comparable to positive controls. These findings underscore the potential of thymol-enhanced coatings in effectively mitigating fungal growth, suggesting their utility as a viable postharvest treatment to enhance quality and extend the shelf life of Cavendish bananas.

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