

Antifungal effect of the edible coating added with cinnamon essential oil (*Cinnamomum verum*) on the shelf life of strawberries (*Fragaria × ananassa*)

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ABSTRACT

Objective: to develop an edible coating with cinnamon essential oil (CEO) to prolong the useful life of strawberries.

Design/Methodology/Approach: We obtained CEO by the distillation method with water and steam, determining the minimum inhibitory concentration through an *in vitro* test with the agar diffusion technique. Also, we determined antifungal activity *in vivo*.

Results: CEO, obtained by the distillation method with water and steam, showed yields of $1.2 \pm 0.3\%$ per extraction cycle. In turn, the *in vitro* test on the minimum inhibitory concentration that CEO presented against the fungus *Rizhopus stolonifer* using the agar dilution technique showed that its growth was completely inhibited at a concentration of 500 ppm. However, the antifungal activity *in vivo* was less effective due to the complex host-antifungal-pathogen system added to the temperature factor. The study at room temperature (27 °C) showed growth of the fungus *Rizhopus stolonifer* on the strawberries with the coating at 100 and 300 ppm on day 3 of treatment. On the contrary, at refrigeration temperature (8 °C), the strawberries coated with the different concentrations of CEO remained in good condition for a longer time, as proved by visual appearance without loss of color which is the appearance of freshness, added to a visually attractive shine.

Limitations/Implications of the study: using cinnamon essential oil (CEO) as an antifungal agent in edible coatings could provide an effective and natural alternative for strawberry preservation. This would potentially impact post-harvest practices. To implement CEO applications, combined with refrigeration, can help to reduce fungal growth and to extend the shelf life of strawberries.

Findings/Conclusions: a synergy of temperature and CEO concentrations should be used to slow the growth of *Rizhopus stolonifera*, to prolong the life time of strawberries, maintaining their appearance without loss of color, which is the appearance of freshness, adding a visually attractive shine.

Keywords: *Rizhopus stolonifer*, edible coating, cinnamon essential oil.

Citation: Colín-Álvarez, Ma. de L., Rentería-Ortega, M., Barrios-Francisco, R., León-Espinosa, E.B., Castañeda-Serna, H.U. & Rafael-Ramírez, J. (2025). Antifungal effect of the edible coating added with cinnamon essential oil (*Cinnamomum verum*) on the shelf life of strawberries (*Fragaria × ananassa*). *Agro Productividad*. <https://doi.org/10.32854/agrop.v18i1.2951>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Dra. Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: June 26, 2024.

Accepted: December 21, 2024.

Published on-line: February 17, 2025.

Agro Productividad, 18(1), January, 2025. pp: 127-135.

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INTRODUCTION

The strawberry (*Fragaria × ananassa*) is a plant belonging to the Rosaceae family. Currently it is considered as a gourmet fruit. Its importance lies on the content of several components such as vitamins E and C, tannins, flavonoids, anthocyanins, catechin, quercetin, kaempferol and organic acids, such as citric, malic, and ellagic, among others. These compounds exert antioxidant properties and help to reduce the risk of cardiovascular

events [1]. However, it is a non-climacteric fruit, highly perishable, with a reduced post-harvest shelf life.

The shelf life of fresh strawberries in refrigeration (0 to 4 °C) is usually about five days. Therefore, conservation constitutes a problem for producers and the industry due to the high speed at which metabolic processes occur. Mainly, a high respiratory rate and the lack of an external barrier to limit the water retention. Other characteristics that limit conservation are the low resistance to mechanical damage, and microbiological attack by fungi—especially *Botrytis cinerea* and *Rizhopus stolonifer*—, causing significant losses during transportation and marketing, and reducing its attributes about flavor, aroma and texture; affecting commercial quality [2].

Food industry is trying to satisfy consumer demands, and it has promoted the development and design of new technologies that allow maintaining and obtaining products with characteristics similar to fresh foods and with a useful life comparable to processed products [5], thus increasing shelf life and offering safe products.

Various food preservation technologies—some of which have been used for a long time—protect fruits from alteration by microorganisms that can be inhibited by refrigeration, reduction of water activity, acidification, and modification of the container atmosphere by non-toxic treatments. Thermal packaging in plastic coatings, surface processing (waxes and paraffin), refrigeration and edible coatings (EC) based on different materials [3].

Concerning ECs, lipids, resins, and polysaccharides such as chitosan have been used for their formulation and design due to their antimicrobial properties. Furthermore, applying antimicrobial agents could prevent or delay the microbial deterioration of food indicates that essential oils such as cinnamon have antimicrobial and antioxidant properties, so they can be considered as a natural preservative. Several studies have also been done on the antifungal and antioxidant properties of essential oils, such as extracts of oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), coriander (*Coriandrum sativum* L.), onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and cinnamon [4].

One of the most effective antifungal agent is cinnamon leaf essential oil (CEO) due to its high antifungal power attributed mainly to its main compound, eugenol, which has a high capacity to stabilize and neutralize free radicals. Its high capacity to inhibit lipid peroxidation induced by reactive oxygen species has been demonstrated [5], so the composition of HCA gives its antifungal and antioxidant properties with the potential to be used as a post-harvest treatment.

Due to the above, the aim of the current study was to determine the antifungal effect of edible coating added with cinnamon (*Cinnamomum verum*) essential oil on the shelf life of strawberries (*Fragaria* × *ananassa*).

MATERIALS AND METHODS

Cinnamon (*Cinnamomum verum*) and strawberry (*Fragaria* × *ananassa*) were obtained from the local market of San Felipe del Progreso, Estado de México. Glycerol (glycerin USP, Azumex, Lot: OBBP76909), tween 80 (Sigma-aldrich), carboxymethylcellulose (NF ingredients, Lot: NFCMC1000), PDA agar (MCD LAB,7041, LOT 70414G053), and microdin disinfectant were also obtained from the local market.

Extraction of cinnamon (*Cinnamomum verum*) essential oil

Cinnamon essential oil (CEO) was extracted according to the methodology reported by Rodríguez & Real (2012) [6] with some modifications. For the distillation technique with water and steam, 250 g of starting material was used of each 2 L of purified water; the sample was placed on the separation grid, and then the distiller lid was placed. Subsequently, the hoses were placed in a container with 15 L of water (one hose was connected to the water pump, and the other was left free).

The essential oil extractor was placed on an electric grill, and then the grill and the water pump were connected to the electric power to obtain the essential oil. At the extractor outlet, a beaker with a capacity of 2 L was placed to collect the decanted hydrolate. The oil obtained was stored in amber bottles, and the extraction yield was determined based on Equation 1.

$$Yield(\%) = \frac{Volume\ obtained\ (mL)}{Sample\ quantity\ (g)} * 100 \quad \text{Equation 1}$$

In vitro assay

The minimum inhibitory concentration (MIC) was performed by dilution in agar. PDA agar was prepared according to the manufacturer's instructions, and a final concentration of 1% (v/v) of Tween-80 was incorporated into the agar medium after autoclaving to improve solubility in cinnamon essential oil (CEO). A series of double concentrations of cinnamon oil was prepared, ranging between 100,300,500 and 600 ppm. Three repetitions were done per treatment in addition to the control. The fungus *Rhizopus stolonifer* was planted in the centre of the Petri dishes as discs with 7 mm diameter taken from a colony with 16 days of culture [7]

Spore collection and quantification

Spores of fungus *Rhizopus stolonifer* were collected from Petri dishes with 16 days of growth, 7.7 mg were weighed and added to 100 mL of sterile water. The solution was stirred at 800 rpm for 45 minutes. Subsequently, a 1:100 dilution was made. A Neubauer chamber was used to calculate the concentration of spores using the four largest squares of the chamber. The sample (suspension of the spores) was applied to the edge of the coverslip, which will enter by capillarity into the space between the slide and the coverslip, filling the chamber. Finally, it was observed under a microscope with a 40X objective, and the spore concentration was calculated according to Equation 2.

$$Concentration / mL = \left(\frac{Number\ of\ spores}{4} \right) (Dilution\ factor) (10,000) \quad \text{Equation 2}$$

Solution preparation

A solution was prepared following the methodology by Mamani & Hernán (2019) [8], with some modifications. 0.4 g of 0.5% carboxymethylcellulose, 1.2 mL of glycerol, 0.8 mL of 1% Tween 80, 76.8 mL of sterile distilled water, and CEO in different concentrations

(100, 300, 500, 700, 900 ppm) were used. Subsequently, the solutions were mixed at 800 rpm for 30 min.

Preparation of edible coatings by immersion

Strawberries were selected by visual examination; those that showed physical damage were discarded (bruises, softening, degradation or rot, visible fungi or non-homogeneous color), and disinfection was made. Fruits were immersed in a solution of purified water with disinfectant micron according to the manufacturer's instructions (amount of disinfectant and disinfection time); fruits were then drained and dried thoroughly.

The edible coating was added according to the methodology reported by Van *et al.* (2023) [9] with some modifications; the strawberries were immersed in the coating for 10 seconds and placed on racks to drain the excess. Subsequently, we applied three sprays of the solution containing the fungus *Rhizopus stolonifer* per fruit (0.60 mL), and they were stored in transparent trays hermetically closed and covered with plastic wrap. Tests were done at room temperature (T_a) (27 °C), by three treatments (100, 300, 500 ppm) with two replicates each and compared to the control. They were inspected in several days (1, 3, 4, 5 and 6) to monitor visual changes in the fruits. On the other hand, strawberries at refrigeration temperature (T_r) (8 °C) were monitored during 16 continuous days.

Experimental design

Experimental data was analyzed using SPSS 13.0 (Statistical Package for the Social Sciences, IBM SPSS, USA). Mean values were calculated and reported as mean \pm SD ($n=3$). One-way analysis of variance followed by the least significant difference test was used on the *in vitro* and *in vivo* antifungal activities of cinnamon essential oil ($p<0.05$).

RESULTS AND DISCUSSION

CEO Extraction yield

The isolated oil fraction of cinnamon presented a pale yellowish to greenish appearance with a strong aroma, with an extraction yield of $1.2 \pm 0.3\%$ per extraction cycle. This agrees with the findings of Kamaliroosta *et al.* (2012) [10] with values of 1.3% of the total weight. However, regarding the extraction cycles of cinnamon essential oil, 15 mL were obtained per 2 kg of raw material. Juarez (2015) [11] reported 6 mL of essential oil from 1 kg of dry cinnamon bark, obtaining a high extraction yield using the same extraction technique in this study. Nonetheless, they differ from those obtained by Vargas, (2019) [12] with a variation of 0.4%. Possibly, the differences are related to the geographical position, the cultivation, the variety of cinnamon, the harvest time, the discrepancy of the method, the climatic and environmental conditions, or the extraction method.

***In vitro* assay**

The minimum inhibitory concentration (MIC) presented by cinnamon oil against the *Rizhopus stolonifer* fungus tested by the agar dilution method is shown in Figure 1. Control showed significant growth on the first day with a growth halo of 18.1 ± 0.03 mm in

diameter, and complete growth on day 3 with a diameter of 80.2 ± 0.1 mm and spores since the fourth day. The 100 ppm treatment showed a behavior similar to the control, with a growth halo of 8.6 ± 0.01 mm in diameter and a difference compared to control of 9.5 mm. According to the values obtained (Table 1), we deduced that MIC in these *in vitro* assays is 500 ppm; growth of *Rizhopus stolonifera* was not observed.

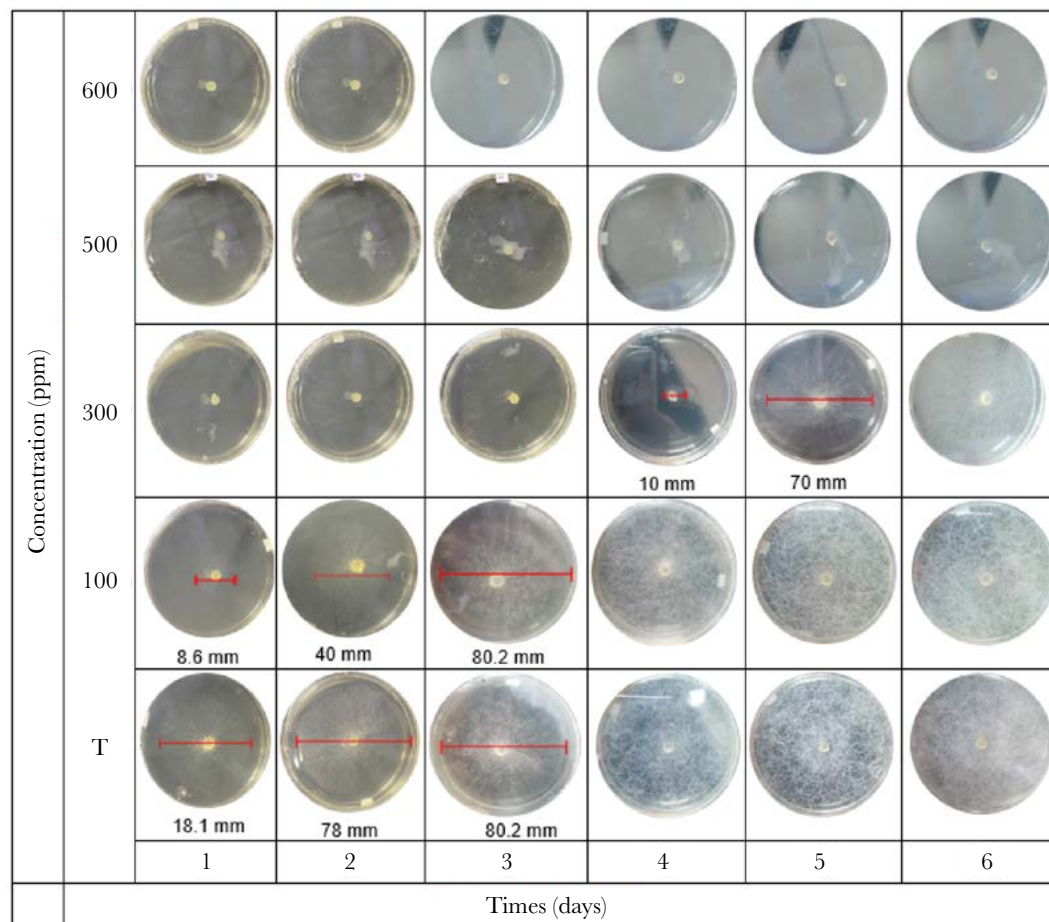


Figure 1. Minimum inhibitory concentration of cinnamon (*Cinnamomum verum*) essential oil on the growth of *Rhizopus stolonifera*.

Table 1. Growth inhibition zone in terms of concentration of cinnamon essential oil.

CEOC (ppm)	Inhibition zone diameter (mm)					
	Day 1	Day 2	Day 3	Day 6	Day 8	Day 10
0.0	18.1 ± 0.03^a	78.0 ± 1.03^b	80.2 ± 0.1^b	80.2 ± 0.09^b	80.2 ± 0.09^b	80.2 ± 0.09^b
100	8.6 ± 0.01^c	40.0 ± 0.8^d	80.2 ± 0.09^b	80.2 ± 0.09^b	80.2 ± 0.09^b	80.2 ± 0.09^b
300	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	10.0 ± 1.01^c	70.0 ± 1.03^d	80.2 ± 0.09^b
500	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c
600	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^c

CEOC, Cinnamon essential oil concentration; Well diameter 10 mm. Different letters show significant difference, expressed as growth inhibition diameter.

Values obtained are similar to those by Ochoa *et al.* (2012) [13] who reported that cinnamon essential oil had an antifungal effect against *Fusarium* spp. at a concentration of $300 \mu\text{g mL}^{-1}$ (300 ppm) on the other hand Xing *et al.* (2010) [14] showed that higher concentrations of cinnamon oil in combinations with clove oil were more effective in inhibiting the growth of *Aspergillus flavus*. While Tzortzakis (2009) [15] reported complete inhibition of spores at concentrations of 500 ppm, which results similar to those obtained in this study.

***In vivo* antifungal activity**

Results of the *in vitro* assays showed that CEO had less antifungal activity when it was tested on fruits at the same concentrations. This is related to the complexity of host-antifungal-pathogen system. Besides, Abdolahi *et al.* (2010) [16] reported that this observation is associated with the alteration of actions in the assay, or structural changes such as hydrolysis, degradation and polymerization of fruits under *in vivo* conditions.

The effects of edible coatings on strawberries —previously infected with *Rhizopus stolonifer*— and the control stored at room temperature (at 27 °C) are shown in Table 2 and Figure 2, respectively. The statistical analysis revealed that at all concentrations of cinnamon essential oil tested, strawberries without the edible coating (control) did not differ significantly ($p > 0.05$) from strawberries coated with a concentration of 100 and 300 ppm since the third day of storage, where 100% of the strawberries showed *Rhizopus stolonifer* growth (Table 2). Therefore, the use of low concentrations in the edible coating will not have an antifungal action in strawberries. On the other hand, at the concentration of 500 ppm, a loss of firmness was observed since day 3; and a spread of fungal infection since day 5, with $50.00 \pm 1.5\%$ of infected fruits. Overall, growth rates increased with temperatures close to the optimum.

On the other hand, when the study was repeated at low temperatures, there was a significant difference. The control showed growth up to fourth day with a growth of

Table 2. Percentage of coated strawberries and uncoated strawberries (control) artificially infected with *Rhizopus stolonifer* and stored at room temperature (27 °C) and refrigeration (8 °C).

Infected strawberries (%) at 27 °C					
CEO (ppm)	Day 1	Day 3	Day 4	Day 5	Day 6
500	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a	50,00±1,5% ^b	50,00±0,00% ^b
300	0,00±0.0% ^a	0,00±0.0% ^a	100,0±0,0% ^c	100,0±0,0% ^c	100,0±0,0% ^c
100	0,00±0.0% ^a	0,00±0.0% ^a	100,0±0,0% ^c	100,0±0,0% ^c	100,0±0,0% ^c
Control	96,67±3,1% ^d	100,0±0,0% ^c	100,0±0,0% ^c	100,0±0,0% ^c	100,0±0,0% ^c
Infected strawberries (%) at 8 °C					
CEO (ppm)	Day 1	Day 4	Day 8	Day 12	Day 16
500	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a
300	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a	0,00±0.0% ^a
100	0,00±0.0% ^a	5,02 ±0.9% ^b	13,33±5,77% ^c	50,00 ±0,0% ^d	60,00±0,0% ^c
Control	0,00±0.0% ^a	15,33±2,52% ^c	30,00 ±0,00% ^f	90,00±0,0% ^g	96,67±2,89% ^h

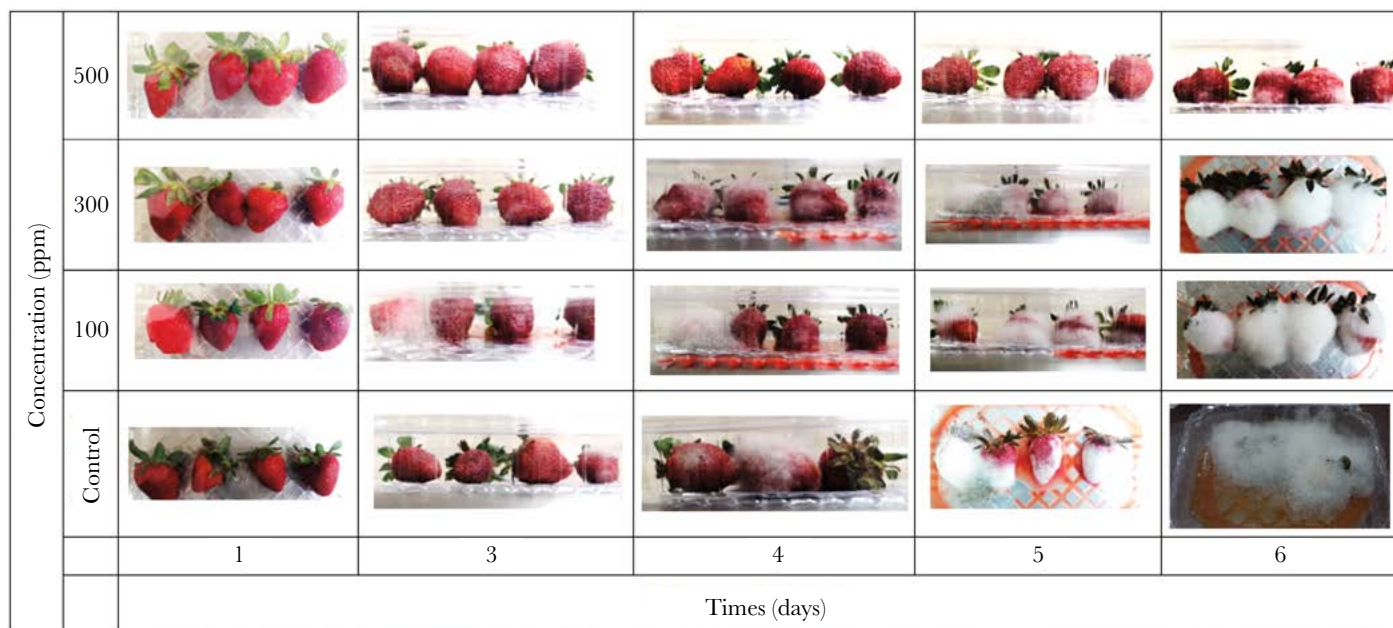


Figure 2. Effect of edible coatings on strawberry (*Fragaria × ananassa*) at room temperature (27 °C).

15.33±2.52%, while on the last day of the study (day 16), the infected fruits increased to 96.67±2.89%. Showing that the severity of the infection decreased starting at 100 ppm, and no growth at 300 ppm. This is an evident effect caused by temperature, that was also reported by Win *et al.* (2007) [17], who mentioned that the antimicrobial activity of essential oils in fruits and vegetables is benefited by a decrease in storage temperature, a higher concentration, and a decreased pH of the food.

While Ojagh *et al.* (2010) [18] demonstrated that cinnamon extract completely inhibited the mycelial growth of the fungi *Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae* at 5.0 g L⁻¹, in banana *in vitro*. Furthermore, Tzortzakis (2009) [15] demonstrated that previously exposing the tomato fruit to 500 ppm of cinnamon for three days, then inoculated it with fungi, reduced the development of lesions caused by *Colletotrichum coccodes* and *Botrytis cinerea*.

According to the results, at low temperatures (8 °C), the percentage of strawberries infected with *Rhizopus stolonifer* and control showed significant differences (p<0.05). Vega-Ríos *et al.* (2014) [19] reported that during fruit storage at low temperatures, there is a decrease in physiological processes and a weakening of the pathogenicity of fungi. This is similar to the observation found in this study at temperatures of 8 °C, where a lower percentage of infection was observed, compared to those strawberries stored at temperatures of 27 °C. With 100, 300 and 500 ppm of CEO there was inhibition of *Rhizopus stolonifer*; we can say that there is a direct relationship between the treatment at refrigeration temperatures and the MIC. In addition, a significant change could be observed in terms of the appearance of the strawberry, as it is shown in Figure 3; since the coated strawberries maintained good visual appearance without loss of color, with appearance of freshness, plus a visually attractive shine.

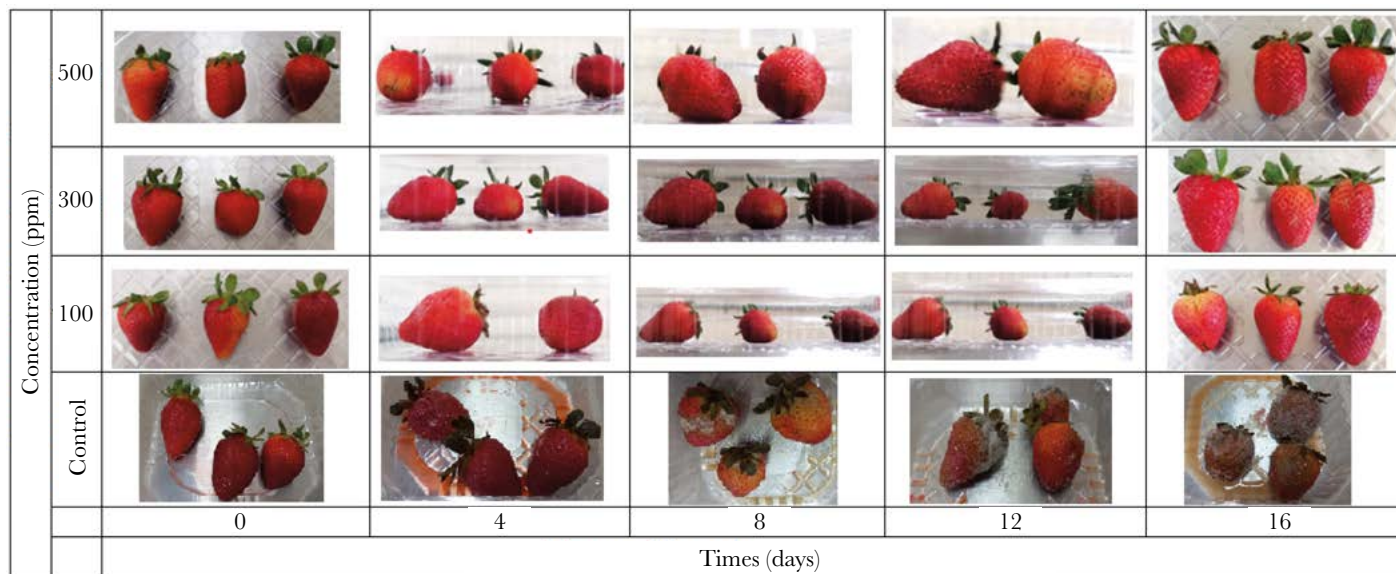


Figure 3. Effect of edible coating on strawberries (*Fragaria×ananassa*) at refrigeration temperature (8 °C).

The previous effect can be attributed to CEO's features; its constituents and hydrophobic properties, since they penetrate the cell membranes of fungi, altering their cellular structure and increasing permeability. Accordingly, dos Santos *et al.* (2012) [20] reported that permeability causes a leak of exhausted ions and other cellular contents, causing fungi cells death. In addition to the above, *Rhizopus* rot is related to temperature, with a maximum fungi growth at 27 °C, similar to what was found in this study.

CONCLUSIONS

The study demonstrated that cinnamon (*Cinnamomum verum*) essential oil effectively inhibits the growth of *Rhizopus stolonifer* both *in vitro* and *in vivo*. The effectiveness of the antifungal action was highly dependent on the essential oil concentration and storage conditions. At room temperature (27 °C), low concentrations (100 and 300 ppm) failed to prevent the proliferation of the fungus, while a concentration of 500 ppm significantly reduced growth up to the third day. However, greater effectiveness of the coating was evident at refrigeration temperatures (8 °C), where coated strawberries showed a notable reduction in fungi infection even after 16 days of storage.

These findings highlight the importance of the synergy between storage temperature and essential oil concentration in controlling *Rhizopus stolonifer* infections. Also preserving the freshness appearance of strawberries, combined effects could extend their shelf life. The use of edible coatings with cinnamon essential oil is a promising strategy for post-harvest preservation of fruits, especially under refrigeration conditions.

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