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Research Paper

Effect Of Glycerol Dosage on Antifungal Performance of Black Cumin Oil–Fortified Edible Coatings on Red Chili (*Capsicum Annuum*)

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Abstract

Edible coatings can stop chili from getting moldy, but it is not often clear how the amount of plasticizer affects antifungal performance. We made a coating out of starch, pectin, and gelatin that was strengthened with 3% black cumin (Nigella sativa) oil and 0, 10, 20, or 30% glycerol (w/w of total biopolymers). The chili that was not coated was the control. Whole red chilies were dip-coated and kept in the dark for 21 days at 25 ± 2 °C and 65 ± 5% RH. On Day 0 and Day 21, fungal occurrence was counted on DG-18 agar with 0.01% chloramphenicol (25 °C, 5–7 d), and vitamin C was measured iodometrically (reported as mg/100 g and retention %). On Day 21, all coatings reduced molds compared to the control, with a precise dose pattern: 0% < 10% < 20% ≈ 30%, resulting in reductions of about 40–48% depending on the dose ($p < 0.05$). The middle range (approximately 20%) provided the most consistent suppression; 30% did not offer any significant benefit over 20%, indicating that higher plasticization levels do not provide as much help. The retention of vitamin C was similar to the microbiological result, with values of about 81% (0%), 85% (10%), and approximately 87% (20–30%), compared to about 70% in the uncoated control. This supports the idea that coatings that remain conformal without excessive surface moisture can both prevent mold growth and slow down oxidative loss during storage. This matrix contains approximately 20% glycerol, a valuable single-ingredient addition that enhances the antifungal effect and maintains the quality of whole chili under dark, cool conditions.

Keywords: *chili, edible coating, glycerol, Nigella sativa, molds*

INTRODUCTION

Fresh chili is susceptible to postharvest deterioration in warm supply chains, with anthracnose and associated molds causing quality degradation and downgrades. Regional surveys and multilocus/phylogenomic analyses in Asia elucidated the *Colletotrichum* complex associated with chili, frequently identifying *C. truncatum, C. scovillei, C. capsici*, and related species on harvested chili, while underscoring the importance of surface-directed interventions at the chili–air interface (de Silva et al., 2019; Hsieh et al., 2022).

Edible coatings add functional substances to the cuticle without interfering excessively during handling. Recent studies and reviews have demonstrated that applying bioactive coatings or similar surface technologies to peppers reduces the number of microbes and facilitates easier storage. However, the results depend on the composition of the matrix and the storage conditions (Tiamiyu et al., 2023; Akbari et al., 2024). Black cumin (Nigella sativa) oil shows considerable promise as a botanical active ingredient. Its bioactive composition, primarily composed of thymoquinone (TQ) and other terpenoids, exhibits potent antibacterial and antifungal properties, making it useful for food and produce (Abdel-Latif et al., 2021; Alberts et al., 2024).

In biopolymer films, glycerol serves as the primary plasticizer, regulating chain mobility, film integrity, and local moisture at the chili–film interface—elements that may affect fungal growth

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dynamics and the longevity of embedded actives. Increasing glycerol from low to moderate concentrations enhances flexibility and conformal contact, although also elevates hygroscopicity and water-vapor permeability at elevated levels (Ballesteros-Martínez et al., 2020; Tarique et al., 2021; Mohammed et al., 2023; Eslami et al., 2023). Maintaining a consistent composition while varying the glycerol dosage enables a clear assessment of how microenvironmental adjustments affect antifungal results on chili. We assessed uncoated and coated chili on Day 0 and Day 21 during ambient storage and measured fungal occurrence. The known light and heat sensitivities of thymoquinone were taken into account while analyzing the effects of storage duration (Karaman, 2020).

LITERATURE REVIEW

This study examines the impact of glycerol dosage (0, 10, 20, 30% w/w of total biopolymers) in a starch–pectin–gelatin coating enhanced with *Nigella sativa* oil (3% w/v) on whole chili, while controlling for all other variables. We measure the amount of fungi that grow after being stored in the dark and at room temperature using established counting methods. At the same time, we report the amount of vitamin C at Day 0 and Day 21 as an independent quality marker that does not depend on anything else. The goal is to find a formulation window that maintains the film's integrity while also controlling moisture at the interface. This way, the coating can be adjusted without compromising its handling properties.

Anthracnose and other molds cause losses in fresh chili that is moved through warm chains. Recent surveys and multilocus/phylogenomic work in Asia have demonstrated that a polyetiologic Colletotrichum complex is present on harvested chili, with the chili–air interface identified as the most critical control point (de Silva et al., 2019; Hsieh et al., 2022). Edible coatings work directly at that interface, and recent studies on peppers show that when film continuity and contact are maintained, there are fewer microbes and the peppers last longer (Tiamiyu et al., 2023; Akbari et al., 2024). *Nigella sativa* oil, particularly thymoquinone (TQ) in conjunction with terpenoids, exhibits extensive antifungal properties; however, TQ is sensitive to light and heat, thus its efficacy is contingent upon the microenvironment established by the film during storage (Abdel-Latif et al., 2021; Alberts et al., 2024; Karaman, 2020).

Glycerol works by altering two interlinked levers that control antifungal outcomes: film integrity/contact mechanics, as well as interfacial transport with active availability. Increasing the plasticizer from low to moderate levels lowers the effective glass transition temperature, reduces micro-cracking, and improves conformal coverage on curved chili surfaces. However, higher doses make the surface more hygroscopic and more permeable to water vapor (WVP), which can make the surface wetter even as flexibility improves (Ballesteros-Martínez et al., 2020; Tarique et al., 2021; Mohammed et al., 2023; Eslami et al., 2023).

RESEARCH METHOD

Materials

Red chili (*Capsicum annum* L.) aged 1–2 days after harvesting was obtained from local market in Condong Catur, Sleman, (Indonesia), sugar palm starch was obtained from local farmers in Borobudur (Magelang, Indonesia), black seed oil (Timur Tengah, Bogor, Indonesia), gelatin (Redman fish gelatin, Ang Mo Kio, Singapore), Low Methoxyl Pectin (LMP) (Campectin 4510, Madrid, Spain), glycerol, microbial media of Potato Dextrose Agar (PDA) were obtained from Milipore (Massachussets, US) and other chemical reagents.

Coating formulation and application

First, 3 g of sugar palm starch was mixed with 50 ml of warm distilled water (60°C) until it dissolved. The mixture was known as starch solution. Second, 3 g of gelatin and pectin (50:50) were mixed together in 50 ml of warm distilled water (60°C) and stirred with a magnetic stirrer until they were completely dissolved, making a pectin-gelatin solution (Fig. 1). Then, 10%, 20%, and 30% glycerol was added as a plasticizer and mixed at 40 °C for an hour. Black seed oil (3%) was added to the solution and stirred in until it was completely dissolved. The solution for the edible coating was now ready to use. According to Heristika et al. (2023), the dipping method was used to put the edible coating solution on the red chili. The red chili samples were first dipped in the coating solution for one minute, then stored at room temperature for 21 days and checked on days 0 and 21. After that, they were drained and left to dry at room temperature.

Total fungal quantification

To examine the fungus presence in the chili, 10 g of each sample was combined with 90 mL of sterile 0.85% NaCl solution. Each combination was agitated vigorously. The fungal cultivation technique was derived from Rahayu et al. (2014). The samples underwent dilution by factors of 10, 100, and 1000. Subsequently, 0.1 mL of the mixture from each dilution was aseptically inoculated onto a Dichloran-18 Glycerol (DG-18) agar plate (Oxoid Ltd., Hants, UK) containing 0.01% chloramphenicol (Merck, Darmstadt, Germany). The plates were subsequently incubated at 25°C for a duration of 5 to 7 days. Following a 5-day incubation period, the fungal colonies were enumerated, and the colony coloration was assessed at the conclusion of the incubation. The incidence of fungal presence was quantified as colony-forming units (CFU) per gram of samples. The percentage reduction normalized to the % colony growth of the control was determined as

Percentage Reduction=(% colony growth in coated chili)/(% colony growth in uncoated chili)

Vitamin C

Vitamin C was quantified using a modified iodine titration method described by Sapei and Hwa (2014). A 100-mL volumetric flask was used to measure out 2.5 g of a uniformly mixed red chili sample. After that, 100 mL of distilled water was added to the flask. The solution was filtered, and the filtrate was put into an Erlenmeyer. Then, 2 mL of a 1% starch solution was added. A 0.01 N Iodine solution was used for the titration. To find out how much vitamin C was in the sample, we measured how much ascorbic acid was in each gram. One milliliter of 0.01 N iodine was equal to 0.88 mg of ascorbic acid.

Data Analysis

The studies were carried out in triplicate, and differences in experimental results were significant at a 95% confidence level ($P < 0.05$).

FINDINGS AND DISCUSSION

Fungal Occurrence in Chili

At Day 21, all coated groups showed lower TYMC than the uncoated control, but the magnitude depended on glycerol dose: most lots followed G10 < G20 ≈ G30 (Table 1).

Table 1. Percentage of the fungal occurrence reduction in the coated chilies

Glycerol Concentration (%)	Fungal Occurence (CFU/g)		% Reduction
	Day 0	Day 21	
0	4.33 ± 0.58[a]	88.33 ± 2.89[c]	39.68 + 3.82[a]
10	4.33 ± 0.58[a]	81.67 ± 2.88[b]	44.28 + 2.08[b]
20	4.33 ± 0.58[a]	77.67 ± 2.52[a]	46.95 + 3.68[c]
30	4.33 ± 0.58[a]	76.33 ± 3.21[a]	47.84 + 4.33[c]
Control	4.33 ± 0.58[a]	146.67 ± 5.77[d]	0

Throughout the treatments, Day-21 fungal loads on coated chilies were significantly reduced compared to the uncoated control, with a percentage reduction of approximately 40–48%. The response monitored glycerol levels: 0% < 10% < 20% ≈ 30%, with the 20–30% range exhibiting the most significant and statistically comparable suppressions (Table 1). This dose–response corresponds with the established function of glycerol as a plasticizer in polysaccharide/protein films: increasing glycerol from approximately 10% to 30% (w/w of biopolymers) diminishes brittleness and enhances conformal contact on curved surfaces, thereby aiding in the maintenance of a continuous barrier at typical infection sites—however, it concurrently increases water absorption and water-vapor permeability (WVP) (Ballesteros-Martínez et al., 2020; Tarique et al., 2021; Mohammed et al., 2023; Eslami et al., 2023). Numerous composite matrices indicate an operating optimum about 20%, where integrity is elevated while moisture accumulation is well regulated (Lau et al., 2022; Weng et al., 2025). Our observation that 30% provided no distinct advantage over 20% aligns with the principle of diminishing returns at elevated plasticizer levels, when increased hygroscopicity may partially counteract the barrier efficacy.

Glycerol decreases the effective glass transition temperature and enhances chain mobility, thereby minimizing micro-cracking and maintaining conformal coverage during manipulation and respiration-induced size fluctuations. Improved contact mitigates moisture "hot spots" and localized nutrient exudates that promote spore germination, consequently inhibiting colony development. Insufficient plasticizer (≈0–10%) leads to film fractures and partial detachment; conversely, excessive plasticizer (≈30%) results in elevated water vapor permeability and surface moisture, fostering a more conducive environment for fungi, despite enhanced flexibility (Ballesteros-Martínez et al., 2020; Eslami et al., 2023).

The coating contained black cumin oil, which has thymoquinone (TQ) and other terpenoids that are effective against a wide range of foodborne yeasts and molds (Abdel-Latif et al., 2021; Alberts et al., 2024). Two factors affect the realized effect: how available it is on the chili's surface and how stable it is when stored. Methodological research indicates that TQ can still be recovered from oil matrices when the surrounding phase permits mobility (Ardhi & Schreiner, 2024). Stability studies show that TQ is sensitive to light and stays better preserved in the dark or in protective structures. Light, on the other hand, speeds up loss. Antioxidant co-components can also improve performance (Karaman, 2020; Ardhi et al., 2025). The storage was dark, and the 20–30% glycerol band likely helped with both contact delivery and microenvironment buffering, which helped keep antifungal activity going until Day 21, which is when we saw the best results at these doses.

The magnitude of reduction we observed (≈0.4–0.5 log equivalent when expressed on a log scale; ~40–48% in % reduction terms) is useful for our purposes and fits with what other studies have found: bioactive coatings and surface technologies on peppers can lower microbial counts and make them last longer in storage when the film stays intact (Tiamiyu et al., 2023; Akbari et al., 2024).

Vitamin C

Given that glycerol-tuned films both reduced fungal occurrence and likely limited oxygen/water activity at the surface, we quantified vitamin C (Table 2) to reflect this quality dimension during storage.

Table 2. Change in vitamin C content in the coated chilies

Glycerol Concentration (%)	Vitamin C (mg/100g)		% Remaining Vitamin C
	Day 0	Day 21	
0	63.67 ± 3.21[a]	51.67 ± 2.89[b]	81.20 ± 3.70[b]
10	63.67 ± 3.21[a]	54.00 ± 1.00[c]	84.92 ± 3.27[c]
20	63.67 ± 3.21[a]	55.33 ± 2.08[c]	86.95 ± 1.20[c]
30	63.67 ± 3.21[a]	55.67 ± 2.31[c]	87.46 ± 1.00[c]
Control	63.67 ± 3.21[a]	44.33 ± 1.15[a]	69.69 ± 1.79[a]

Vitamin C levels support the antifungal pattern. The contents on Day 0 were deliberately made the same for all groups (about 63–64 mg/100 g), which showed that the starting chili was the same and that there was no analytical bias from the iodometric method. After 21 days, all of the coated chilies had more vitamin C than the uncoated control. The retention went up from about 81% at 0% glycerol to about 85% at 10% and about 87% at 20–30%, while the uncoated control only had about 70%. The same 20–30% glycerol band that caused the most fungal growth also kept the most vitamin C.

This co-movement fits with the microenvironment that the coating makes. Adequate plasticization enhances film integrity and conformal contact, mitigating micro-cracks and moisture "hot spots" conducive to spore colonization; it also regulates oxygen and water vapor exchange at the chili–air interface (Ballesteros-Martínez et al., 2020; Tarique et al., 2021; Mohammed et al., 2023; Eslami et al., 2023). A drier, better-sealed surface not only stops mold growth (DG-18 counts) but also slows down the loss of ascorbic acid through oxidation, which is why the Day-21 vitamin-C values are higher (Tiamiyu et al., 2023).

The plateau from 20% to 30% glycerol mirrors the antifungal endpoint: added plasticizer past ~20% brings diminishing returns. At higher doses, the matrix becomes more hygroscopic and WVP increases, which can partly offset the benefits of flexibility (Ballesteros-Martínez et al., 2020; Eslami et al., 2023). That the 0%-glycerol coating still outperformed the uncoated control indicates that even a brittle film with *Nigella sativa* oil can reduce oxidative and microbial stress, but mechanical fragility likely reduced coverage during storage, explaining its lower retention relative to 10–30%.

Finally, the vitamin-C trend makes sense with how the active works. Thymoquinone (TQ) in N. sativa oil can help fight fungi, but it is sensitive to light and heat. To keep it functional, films that stay intact and storage in the dark are important (Karaman, 2020; Ardhi & Schreiner, 2024; Ardhi et al., 2025). In practice, adjusting glycerol to about 20% strikes a good balance between strong mold suppression on DG-18 and high vitamin-C retention, without the risk of moisture buildup that comes with higher plasticizer levels.

CONCLUSIONS

Adjusting glycerol in the starch–pectin–gelatin coating fortified with Nigella sativa oil decisively shaped outcomes after 21 days: all coated chilies had less fungus on DG-18 than the uncoated control, with a clear dose pattern (0% < 10% < 20% ≈ 30%) and ~40–48% reductions depending on dose. Quality followed the same trend, with Day-21 vitamin C retention rising from ≈81% (0%) to ≈85% (10%) and ≈87% (20–30%) versus ≈70% in the control. This shows that the coatings that worked best at stopping molds also best preserved the labile nutrient. Taken together, the results suggest that ~20% glycerol is a good choice that keeps the film intact and covers evenly without the moisture pickup seen at higher plasticization, providing consistent antifungal and nutritional benefits when stored in a dark, ambient environment.

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