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Biocompatible Nanopolymer Coating Based on Chitosan Nanoprecipitation Containing Guava Oil to Control Fruit Rot, Enhance Defense-Related Enzymes, and Quality of Chili Pepper

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ABSTRACT

This study evaluates the efficacy of chitosan nanoprecipitation loaded with guava leaf essential oil as a sustainable alternative to synthetic preservatives for reducing postharvest deterioration in *Capsicum frutescens* (chili pepper). Essential oils extracted from *Psidium guajava* leaves via hydrodistillation were analyzed by GC-MS, identifying *β*-caryophyllene (22.44%) as the major constituent. Nanoparticles were synthesized via solvent displacement (NP with guava oil and NNP without oil) and nano-elaboration (NC with guava oil and NNC without oil), and characterized by DLS and TEM, confirming stable sizes and zeta potentials. Antifungal activity of NP and NC was tested against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, both pathogenic to chili. Complete fungal inhibition occurred in vitro by Day 5, whereas in vivo application significantly reduced disease incidence and prolonged shelf life up to 28 days. Treated fruits showed reduced weight loss, better firmness, lower electrolyte leakage, enhanced antioxidant activity, higher phenolic content, and reduced enzymatic browning. Encapsulation and release studies demonstrated controlled diffusion of guava oil, enhancing antimicrobial efficacy. Elevated activities of *β*-1,3-glucanase, phenylalanine ammonia lyase, and polyphenol oxidase indicated improved resistance to oxidative stress and microbial attack. Toxicity assays using *Artemia salina* confirmed formulation safety. These findings highlight chitosan nano-encapsulated guava oil as a safe eco-friendly biopreservative for chili peppers.

1 | Introduction

Fruits and vegetables, exemplified by *Capsicum frutescens*, boast a wealth of essential nutrients, such as vitamins and antioxidants and amino acids, pivotal for digestive health. Despite their nutritional value, the commercial viability of these fruits is hampered by their limited shelf life as documented by several studies (Adeogun et al. 2017, 2018a, 2018b; Breda et al. 2017; Liu et al. 2016; Luís et al. 2016). The constrained longevity of fruits and vegetables is primarily attributed to inadequate postharvest handling and storage infrastructure, leading to a rapid decline in quality exacerbated by microbial contamination (Breda et al. 2017). In response to this challenge, the widespread use of synthetic preservatives has been discouraged due to their recognized health risks (Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Gómez y Gómez 2017).

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Capsicum frutescens, a perennial member of the Solanaceae family, thrives in tropical and subtropical regions and is extensively cultivated. Renowned globally for its pungency, distinct flavor profile, and versatility in culinary applications, pepper serves as both a spice and a natural preservative to prolong the shelf life of fresh and processed vegetables (Saleh et al. 2018). Its popularity stems from not only its sensory attributes but also its rich nutritional composition essential for human health (Zou et al. 2015). However, challenges persist in maintaining the safety and quality of these fruits throughout the supply chain, from harvest to consumer, due to inadequacies in harvesting practices, postharvest management, storage, processing, packaging and transportation, and marketing strategies. These inadequacies accommodate the incursion of microorganisms, causing diseases, such as fruit rots, in chili peppers.

The imperative to mitigate the impact of fruit rots caused mostly by pathogenic fungi on postharvest losses of fruits and vegetables has been extensively documented, prompting the exploration of diverse methodologies to suppress their pathogenic effects and notably enhance the shelf life of fruits such as chili pepper. Hong et al. (2015) elucidated the efficacy of synthetic nonsystemic fungicides, including folpet, benomyl, chlorothalonil, and trifloxystrobin, in mitigating diseases, such as anthracnose disease, in chili pepper. However, escalating safety concerns associated with certain synthetic fungicides and the emergence of resistance among pathogens underscore the preference for natural antimicrobials and irradiation in controlling pathogenic diseases in chili pepper.

Lippia javanica, *Artemisia afra*, *Cassia abbreviata*, *Psidium guajava*, and *Terminalia sericea* have garnered recognition for their antimicrobial properties, substantiated by their demonstrated efficacy against food spoilage microbes (Adeogun et al. 2018a, 2020; Shai et al. 2013). Essential oils derived from these botanical sources are esteemed for their safety profile, rendering them viable candidates for food preservation purposes (Khorshidian et al. 2017).

The volatility of essential oils may limit their efficacy on fruits and vegetables, but this can be addressed by incorporating them into edible coatings (Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Corona-Rangel 2017). The advancement of edible coatings, such as Chitosan, integrated with antimicrobials, such as essential oils, has introduced nanoprecipitation and nanoencapsulation techniques, resulting in coatings with nanometer-scale thickness. This submicron scale enables alterations in gas transport properties and controlled release of natural compounds, enhancing mechanical strength, transparency, functionality, and antimicrobial effectiveness.

Nanosystems, owing to their higher surface area per mass compared to larger particles of the same composition, exhibit enhanced stability and biological activity. Their nanoparticle size facilitates the incorporation of hydrophobic compounds with active ingredients without compromising the visual appeal of food samples, thereby extending their shelf life (Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Corona-Rangel 2017; Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Gómez y Gómez 2017).

Employing natural antimicrobials to combat pathogens in fruits and vegetables, such as chili pepper, can activate resistance genes or induce protection against diseases. Utilizing essential oils from plant parts, such *as P. guajava* leaves, may stimulate gene expression related to disease resistance in *Capsicum frutescens.* This leads to interactions between antimicrobial agents and pathogen molecules, ultimately disrupting their structure. Furthermore, antimicrobial agents can interfere with enzymes, plasmids, and proteins essential for disease proliferation (Deng et al. 2015; Iqbal et al. 2016). *P. guajava,* a plant with antimicrobial properties commonly known as guava and belonging to the Myrtaceae family, is a small tree with shallow roots and smooth bark. Leaves are aromatic, opposite, and elliptic to oblong-lanceolate. Flowers are white, fragrant, with 4–5 petals. Fruits vary in shape, weighing 25–500 g, with sweet pulp and seeds (Medina and Herrero 2015; Pereira et al. 2023).

The effectiveness of natural antimicrobials in extending the shelf life by hydrolyzing fungal cell wall polymers has been demonstrated, potentially enhancing defense mechanisms against fruit rot, as established by Bill et al. (2017). Previous studies have shown that natural antimicrobials can activate defense-related enzymes, preventing diseases, such as anthracnose, in fruits such as avocados (Bill et al. 2016, 2017; Obianom et al. 2019). However, there is limited research on the use of chitosan nanocapsules loaded with essential oils from *P. guajava* leaves to combat pathogens, affect defense-related enzymes in *C. frutescens*, and enhance the quality of *C. frutescens* fruits. Nevertheless, the safety of both chitosan and guava oil has been well-documented in the literature, and they are generally regarded as safe (GRAS).

2 | Materials and Methods

2.1 | Materials

Fresh plant samples for the extraction of the essential oils were collected from the wild, whereas *C. frutescens* was obtained with permission from a number of designated farms (Adeogun et al. 2018a, 2018b). The edible coatings and reagents used in this study were obtained from HiMedia Laboratories Private Limited.

2.2 | Extraction of the Essential Oil From the Test Plant

The dried leaves of *Psidium guajava* were subjected to hydrodistillation using a Clevenger-type apparatus based on the modification of the method described by Elhawary et al. (2024). For each extraction, 3000 g of dried leaves were placed in the distillation flask, covered with deionized water, and heated to boil gently for 4 h. During this process, the essential oils vaporized and condensed in the cooled receiving arm, separating into an aqueous and essential oil layer. The essential oil layer was collected and transferred to an amber-colored glass vial. The average yield from three independent extractions was 1.4 mL of essential oil. The collected essential oils were

immediately sealed in vials and stored at 4°C until further analysis (Elhawary et al. 2024).

2.3 | Characterization of the Essential Oils

The volatile components of guava leaf essential oil were analyzed using GC–MS machine (Perkin Elmer Instruments, Norwalk CT 06859, United States of America). Helium was the carrier gas with a 1:200 split ratio. Mass spectra were acquired from 35 to 500 m/z and processed with the TurboMass 6.1.0 software. Individual components were identified by matching their mass spectra against the NIST library. Relative abundances were quantified using the peak area integration method, expressing each component's peak area as a percentage of the total (Matche and Adeogun 2022).

2.4 | Chitosan Nanoprecipitation Containing Guava Oil Preparation

Two methods, the solvent displacement method and the nanoelaboration method, were used to prepare chitosan nanoprecipitation containing guava essential oil. The preparation of the chitosan nanoprecipitation containing guava oil materials was based on the methods adopted by Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Gómez y Gómez (2017) and Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Corona-Rangel (2017).

2.4.1 | Solvent Displacement Method

Chitosan nanoprecipitation containing guava oil preparation were prepared using the solvent displacement method described previously by Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Gómez y Gómez (2017) and Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Corona-Rangel (2017). The solvent phase was prepared by dissolving chitosan (0.05% w/v) in 2% v/v acetic acid. The nonsolvent phase consisted of 5% guava essential oil in methanol. A volume of 2.5 mL of the solvent phase was added dropwise to the nonsolvent phase (40 mL) under moderate stirring using an ultrasonicator. The resulting aliquot was transferred to a rotary evaporator (Buchi, England) and subjected to solvent evaporation at 40°C and 50 rpm rotation speed. The final nanoparticle suspension was collected and stored under refrigeration at 4°C until further use.

2.4.2 | Nanoelaboration Method

Chitosan nanoprecipitation containing guava oil preparation were prepared using the nanoelaboration method described previously by Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Gómez y Gómez (2017) and Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Corona-Rangel (2017). Lecithin (30 mg) was dissolved in 0.5 mL of ethanol to form the organic phase. Guava essential oil (0.125 mL) and 9.5 mL of acetone were added to the organic phase. The aqueous phase consisted of chitosan (0.5% w/v) dissolved in 20 mL of MilliQ water. The organic phase was incorporated into the aqueous phase under constant agitation for 10 min using an ultrasonicator. The resulting solution was transferred to a rotary evaporator (Buchi, England) and subjected to solvent evaporation at 40°C and 50 rpm rotation speed to obtain a final suspension volume.

2.5 | Physicochemical Characterization of Chitosan Nanoprecipitation Containing Guava Oil

2.5.1 | Dynamic Light Scattering

Dynamic light scattering (DLS) technique was employed to determine the zeta potential and particle size of chitosan nanoprecipitation containing guava oil prepared via solvent dispersion and nanoelaboration methods. Measurements were conducted using a Zetasizer Nano-ZS (Malvern Instruments). To initiate the analysis, 1 mL of the nanoprecipitated oil solution was injected into a Zetasizer Nano Z foldable capillary Zeta cell (Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Gómez y Gómez 2017; Sotelo-Boyás, Correa-Pacheco, Bautista-Baños, and Corona-Rangel 2017).

2.5.2 | Determination of Loading Capacity and Encapsulation Efficiency

The loading capacity (LC) of guava oil in the nanoparticles or nanocapsules was determined by a spectrophotometric method based on methods adopted by Herculano et al. (2015). A calibration curve was constructed by measuring the absorbance of the concentrations of guava oil in ethanol at 210 nm using a UV-visible spectrophotometer. The dried nanosample (10 mg) was weighed and dissolved in ethanol, and the absorbance was measured at 210 nm. The concentration of loaded guava oil was determined from the calibration curve, and the loading capacity was calculated using the following equation:

$$\mathrm{LC}(\%) = \frac{\text{Weight of loaded guava oil}}{\text{Weight of nanoparticles or nanocapsules}} \times 100 \quad (1)$$

The encapsulation efficiency of the nanosuspension was centrifuged at 20,000g based on method adopted by Herculano et al. (2015) and separated at 4°C for 30 min for the separation of encapsulated guava oil from the free (nonencapsulated) guava oil. The supernatant containing the free guava oil was collected, and the absorbance was measured at 210 nm using the same calibration curve as for the loading capacity determination. The weight of free guava oil was calculated from the absorbance value. The encapsulation efficiency was then calculated using the following equation:

$$\mathrm{EE}\ (\%) = \frac{\text{Weight of initial guava oil} - \text{Weight of free guava oil}}{\text{Weight of initial guava oil}} \times 100 \quad (2)$$

2.5.3 | Transmission Electron Microscopy of Chitosan Nanoprecipitation Containing Guava Oil

Nanocapsules were analyzed via TEM for morphology and size distribution. A nanocapsule suspension was pipetted onto a copper grid and air-dried. Imaging was carried out with a JEOL JEM-2100 LaB6 microscope (200 kV), and digital micrographs were captured using an Orius 830 CCD camera. ImageJ software was used to determine particle size and distribution by measuring ≥ 100 nanocapsules from different TEM grid regions (Matche and Adeogun 2022).

2.5.4 | In Vitro Release Kinetics

The in vitro release kinetics of guava oil from the chitosan nanoprecipitation were studied based on the method adopted by Herculano et al. (2015) and Matche and Adeogun (2022) using a membrane diffusion method. The nanoprecipitation (100 mg) was dispersed in 10 mL distilled water and separated through a membrane filter. The membrane containing the nanoprecipitation was immersed in a release medium of 200 mL distilled water maintained at 37 ± 1°C and agitated at 100 rpm. Aliquots were withdrawn from the release medium at predetermined intervals and replaced with fresh distilled water. The aliquots were analyzed using a Shimadzu UV-1800 UV-visible spectrophotometer to determine the concentration of released guava oil. The experiment was conducted in triplicate, and the percentage of guava oil released at each time point was calculated based on the amount of encapsulated oil. To describe the release kinetics and mechanism, the in vitro release data were analyzed using various kinetic models, including zero-order, first-order, and Higuchi models (Equation 3).

$$\begin{array}{cc} Q_t = k_0 t & \text{(zero - order)} \\ ln\ Q_t = ln\ Q_0 + k_1 t & \text{(first - order)} \\ Q_t = k_H t^{0.5} & \text{(Higuchi model)} \end{array} \quad (3)$$

The cumulative quantity of oil released at a given time point (t) is represented by the variable Q_t, whereas Q_0 denotes the initial amount of oil incorporated into the chitosan nanoprecipitate. The rate constants associated with the zero-order, first-order, and Higuchi kinetic models are denoted as k_0, k_1, and k_H, respectively.

2.6 | Evaluation of Antifungal Activity of Chitosan Nanoprecipitation Containing Guava Oil

2.6.1 | Isolation and Identification of Pathogenic Fungi Associated With *C. frutescens*

Fungi were isolated from infected chili pepper following a modified method of Matche and Adeogun (2022), where the fruits were thoroughly washed with double-distilled water and surface-sterilized using 70% ethanol for 30 s, followed by rinsing with double-distilled water, small cubes were excised from the fruit lesions and placed on potato dextrose agar (PDA) plates, supplemented with 100 mg of chloramphenicol dissolved in 20 mL of ethanol before pouring into 90 mm Petri dishes, emerging fungal colonies that were incubated in a BOD incubator were repeatedly subcultured using the hyphal tip technique until pure cultures were obtained, and the isolates were identified by microscopic examination and comparison with relevant literature.

2.6.2 | Pathogenicity Test

Pathogenicity test based on Matche and Adeogun (2022) adjusted method were conducted on yet to fully ripen chili fruits to determine if previously isolated fungi caused infections. Mycelial plugs from actively growing cultures were aseptically excised and inoculated onto wounded fruits. Inoculation sites were covered with sterile cotton and sealed with Parafilm for high humidity. Fruits were incubated at 25°C for 48 h, then transferred to 24 ± 2°C for 5 days with Parafilm removed. On day 6, fruits were assessed for symptoms. Lesions from infected and control fruits were surface-sterilized and plated on PDA to permit fungal outgrowth. Plates were incubated at 25°C for 5 days. Morphological and microscopic examination confirmed reisolated fungi matched the original inoculated isolates, indicating their pathogenicity on chili fruits.

2.6.3 | Morphological Characterization by Scanning Electron Microscopy

Fungal isolates were prepared for scanning electron microscopy (SEM) analysis. Mycelia were fixed on grease-free slides with 3% glutaraldehyde, washed in cacodylate buffer, and dehydrated through an ethanol series (30%–100%, 1h each). Critical point drying with liquid CO_2 replacement prevented sample collapse. Dried samples were mounted on stubs, sputter-coated with gold/platinum, and examined using SEM (LEO 435VP). The SEM electron beam scanned the conductive-coated samples, generating high-resolution images to visualize the three-dimensional morphology of fungal structures, such as mycelia, spores, and reproductive bodies, in intricate detail (Matche and Adeogun 2022; Tesfay et al. 2017).

2.6.4 | In Vitro Antifungal Activity of Chitosan Nanoprecipitation Containing Guava Oil

The in vitro antifungal potential of nanocapsules against fungal pathogens was evaluated according to the method described by Matche and Adeogun (2022). Potato dextrose agar (PDA) was supplemented with the chitosan nanoprecipitation containing guava oil prepared using the two methods, and 20 mL of the amended medium was dispensed into 90 mm Petri dishes. Mycelial discs (3 mm diameter) were excised from the actively growing edges of fungal isolate cultures obtained during pathogenicity screening and inoculated onto the chitosan nanoprecipitation containing guava oil-supplemented PDA plates. Control plates were prepared by amending PDA with distilled water and inoculating with the pathogen. Inoculated plates were incubated at 28°C for 7 days, and the radial growth of fungal cultures was measured daily using a ruler.

2.6.5 | In Vivo Antifungal Activity of Chitosan Nanoprecipitation Containing Guava Oil

The in vivo antifungal efficacy of chitosan nanoprecipitation containing guava oil was evaluated by employing the modified method adopted by Matche and Adeogun (2022). Chili pepper fruits exhibiting no symptomatic manifestations were subjected to surface sterilization by immersion in 70% ethanol for 30 s, followed by rinsing with double-distilled water (ddH_2O). The fruits were subsequently air-dried in a laminar airflow chamber for 30 min before being immersed in a control treatment (ddH_2O) and various chitosan nanoprecipitation containing guava oil: NNP, NNC, NC, and NP. After drying for 30 min, the fruits were inoculated with the pathogen by making a 5 mm wound and introducing the fungal inoculum. The incidence of disease symptom development (percentage) was quantified for each treatment group.

2.7 | Quality Assessment of Chili Pepper Enhanced With Chitosan Nanoprecipitation Containing Guava Oil

Following the adjusted methods outlined by Adeogun et al. (2023) and Matche and Adeogun (2022), fresh chili pepper fruits underwent a two-stage rinsing procedure utilizing sterile distilled water. After the rinsing process, distinct groups of fruit samples were immersed in solutions containing chitosan nanoprecipitation with guava oil through solvent dispersion and nanoelaboration methods separately, chitosan nanoprecipitation through solvent dispersion and nanoelaboration methods separately, and a control solution without any nanoprecipitation materials under aseptic conditions. Following a 2 min immersion interval, the samples were retrieved and individually housed in unsealed polypropylene containers, allowing for air-drying for 30 min at ambient room temperature under a fume hood. The materials were subsequently stored at room temperature with a relative humidity of 76% for a duration of 28 days, during which quality parameter assessments were performed on days 0, 7, 14, 21, and 28.

2.7.1 | Weight Loss

To determine the physiological weight loss, the test chili fruits were obtained and the initial mass was recorded using a precision weighing balance (Hellog WT1003 N). The fruits were then stored under specified environmental conditions. After the designated storage duration, the mass of the fruits was remeasured. The weight loss was quantified as a percentage of the initial mass by employing the formula below:

$$\text{Weight Loss (\%)} = \frac{\text{Initial Mass} - \text{Final Mass}}{\text{Initial Mass}} \times 100 \quad (4)$$

2.7.2 | pH Measurement

Chili juice were squeezed out from the chili pepper fruit, and the pH meter (Oakton pH 5+) was calibrated according to the manufacturer's guidelines. Subsequently, the pH electrode was immersed in the extracted juice, and the pH reading was recorded (Adekunle et al. 2021).

2.7.3 | Total Soluble Solids (TSS)

A small quantity of juice was extracted from chili pepper fruit, and a handheld refractometer (Erma RHB-32ATC) was used to determine the total soluble solids (TSS) according to the manufacturer's instructions. A drop of the extracted juice was placed onto the refractometer's prism surface, and the TSS value was displayed on the refractometer based on the result obtained from the graduation boundary seen through the eyepiece. The results were then expressed in °Brix units (Adeogun et al. 2023).

2.7.4 | Total Phenolic Content

A stock solution of gallic acid was prepared by weighing 0.5 g of gallic acid and adding it to a 10 mL volumetric flask, dissolving the gallic acid in 10 mL of methanol and making up the solution to 100 mL, followed by the preparation of a calibration curve by diluting the stock solution with 0–100 mg/L gallic acid. In a test tube, 0.5 mL of extracted chili pepper juice was mixed with 2.5 mL of a 10-fold diluted Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution, the tube was covered with aluminum foil and allowed to react for 30 min at room temperature (27°C), and the absorbance of the solution was measured at a wavelength of 765 nm using a single-beam UV-Visible spectrophotometer. The samples were prepared in triplicate, and the mean absorbance values were recorded. A gallic acid calibration curve was constructed using the absorbance values of the standard solutions, and the total phenolic content of the juice was calculated using the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh fruit weight (Abduh et al. 2023).

2.7.5 | Antioxidant Activity (DPPH)

A 0.1 mM DPPH solution was prepared by dissolving DPPH (Sigma-Aldrich, USA) in methanol. In a test tube, 0.5 mL of the chili pepper fruit juice was mixed with 3 mL of the DPPH working solution and the mixture was incubated in the dark for 30 min at room temperature. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (Azotta 1200). The DPPH radical scavenging activity was quantified as a percentage using this Equation (5):

$$\text{\% DPPH Antioxidant Capacity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100\% \quad (5)$$

where the control was the DPPH solution without the chili pepper fruit juice, and the sample was the mixture of chili pepper fruit juice and DPPH solution (Dzięcioł et al. 2023; F. Wang et al. 2023).

2.7.6 | Firmness

The Universal Texture Measuring System (Lloyd, LR-5K) was calibrated according to the manufacturer's guidelines and equipped with a 5 mm cylindrical probe and determined based on the modification of the methods of Llorca et al. (2023). The fruit was positioned on the base of the texture analyzer, and the firmness was measured by compressing the fruit with the cylindrical probe. The firmness value was recorded in Newton (N).

2.7.7 | Water Activity (a_w)

The water activity meter (Novasina) was calibrated following the manufacturer's instructions, and water activity of chili pepper was determined based on the method described by Dufera et al. (2023) and Tarahi et al. (2023). The fruit sample was prepared according to the manufacturer's guidelines and placed inside the measurement chamber of the water activity meter. The water activity value displayed by the meter was recorded.

2.7.8 | Electrolyte Leakage (EL)

Five centimeter diameter discs were excised from the fruit sample and immersed in test tubes containing 25 mL of distilled water. The samples were agitated continuously for 5 h. After the 5 h incubation, the initial electrolyte leakage (EL_i) of the solution was measured using a conductivity meter (model HI9835, Hanna Instruments). The fruit discs in the distilled water were then boiled for 20 min, and after boiling, the final electrolyte leakage (EL_f) of the solution was measured using the same conductivity meter. The electrolyte leakage (EL) was calculated using the formula:

$$EL = \frac{EL_f - EL_i}{n} \quad (6)$$

where EL_f is the final electrolyte leakage after boiling, EL_i is the initial electrolyte leakage after the 5 h incubation, and n is the number of test chili peppers evaluated (Huang et al. 2021).

2.8 | Effect of Chitosan Nanoprecipitation Containing Guava Oil on Antioxidant Enzymatic Activities of Chili Pepper Fruits

2.8.1 | Preparation of Extracts for Enzymatic Activities

Twenty chili pepper fruits weighing 1 g were collected from a region about 2 mm away from the area where the fruit had been wounded. The fruit samples were homogenized using the proper buffer solutions, and then the mixtures were centrifuged at 15,000 g for 30 min at 4°C to extract the supernatants. These extracted samples were then evaluated every 5 days over a 20-day storage period (Ashfaq et al. 2021; Renganathan et al. 2020; Soleimani, Mostowfizadeh-Ghalamfarsa, Ghanadian, et al. 2024).

2.8.2 | β-1,3-Glucanase

The crude enzyme extract (62.5 μL) was mixed with 62.5 μL of laminarin. This mixture was then incubated at 40°C for 10 min. The reaction was stopped by adding 375 μL of dinitrosalicylic acid and heating the mixture in a boiling water bath for 5 min. The resulting solution was diluted with 4.5 mL of distilled water, and the absorbance was measured at 500 nm. A blank sample was prepared by adding the crude extract to laminarin without any incubation time. The enzyme activity was expressed as the microgram equivalent of glucose produced per minute per gram of protein.

2.8.3 | Polyphenol Oxidase (PPO)

1.0 mL of 0.1 M sodium phosphate buffer at pH 6.5, 1 mL of 0.01 M L-tyrosine in HCl, and 0.9 mL of distilled water were added to a cuvette. Then, 100 μL of the fruit extract were added, and the absorbance was measured at 280 nm. The change in absorbance was monitored every 30 s for 2 min, and the PPO activity was expressed as the change in absorbance per minute per gram of fresh leaf tissue.

2.8.4 | Phenylalanine Ammonia-Lyase (PAL)

The assay mixture consisted of 62.5 μL of the enzyme extract, 800 μL of sodium borate buffer, and 700 μL of 12 mm phenylalanine. This mixture was incubated at 40°C in a water bath for 1 h. The reaction was stopped by adding 200 μL of 5 N hydrochloric acid, and then 0.5 mL of 1 M trans-cinnamic acid was added. The absorbance was measured at 290 nm.

2.9 | Toxicity Mechanism of Chitosan Nanoprecipitation Containing Guava Oil by Using Brine Shrimp (*Artemia salina*) as Model

Brine shrimp (*Artemia salina*) cysts were obtained from an aquaculture store in Nigeria, stored at 4°C, and hatched by incubating 500 mg for 24 h at 28°C under a 16 h light and 8 h dark cycle in continuous aerated 30% salinity artificial seawater (ASW). Newly hatched nauplii (24–48 h) were separated from unhatched cysts based on phototaxis and transferred to beakers containing ASW using a Pasteur pipette based on previous methods established by Pinheiro et al. (2024) and Qwarse et al. (2024).

Acute toxicity of chitosan nanoprecipitation with guava oil through solvent dispersion and nanoelaboration methods separately, chitosan nanoprecipitation through solvent dispersion and nanoelaboration methods separately, were tested on the nauplii at various concentrations (1, 10, 100, 500, and 1000 μg/mL). Nauplii were also exposed to negative control solution (ASW) and positive control solution (Potassium dichlomethane, $K_2Cr_2O_7$). The experiment was conducted in 90 mm Petri dishes at 24°C under 12 h light/12 h dark photoperiod.

2.10 | Data Analyses

The datasets were subjected to analysis of variance (ANOVA), in which the means of the test samples were evaluated using Tukey's honest significant difference test at a significance level of 5%. The statistical analyses were conducted using the SPSS software (version 23). The datasets were similarly analysed using a centred second-order polynomial (quadratic) model. Afterward, the dataset was subjected to curve-fitting using nonlinear regression techniques via GraphPad Prism (8. 4. 3.).

3 | Results

3.1 | Essential Oil Characterization

The chemical composition of essential oil from the leaves of *Psidium guajava* (guava) was analyzed using gas chromatography–mass spectrometry (GC–MS). The GC–MS analysis results are presented in Table 1, which reveals 12 major constituents of the guava oil.

Table 1 revealed that β-caryophyllene (22.44%) was the constituent with the highest composition, whereas limonene and α-humulene with 9.24% and 4.76%, respectively, showed considerable compositions after the β-caryophyllene as shown in Table 1.

3.2 | Nanoprecipitation Characterization

3.2.1 | Particle Size Analysis

The particle size distributions of the chitosan nanoencapsulate (NNP) and chitosan nanoencapsulated guava oil (NP) produced through the solvent displacement method are illustrated in Figure 1a,b, respectively. The NNP exhibited a bimodal particle size distribution spanning the range of 50–1000 nm, with an average particle size (z-average) of 443.50 nm (Figure 1a).

In comparison, the NP displayed a broader multiple-peaked particle size distribution ranging from 2 to 6000 nm, with a z-average particle size of 1064 nm (Figure 1b).

Figure 2a,b present the intensity-based particle size distributions for the chitosan nanoparticles (NNC) and chitosan encapsulated guava oil (NC) produced through the nanoelaboration method. The NNC sample exhibited a multipeaked particle size distribution ranging from 2 to 7000 nm, with a z-average of 576.9 $\pm$ 316.6 nm (Figure 2a). Similarly, the NC sample showed a multipeaked distribution spanning from 30 to 8000 nm, with a z-average of 1687 $\pm$ 20.68 nm (Figure 2b).

Table 2 depicts the zeta potential analyses of chitosan nanencapsulate (NNP), chitosan nanoencapsulated guava oil (NP) produced through the solvent displacement method and and chitosan nanencapsulate (NNC), chitosan nanoencapsulated guava oil (NC) via nanoelaboration method. NNP exhibited a zeta potential of 27.1 $\pm$ 3.71 mV, whereas NP had a zeta potential of 7.34 $\pm$ 5.75 mV. Furthermore, NNC displayed a zeta potential of 50.2 $\pm$ 6.83 mV, and NC exhibited a zeta potential of 58.8 $\pm$ 7.47 mV (Table 2).

3.2.2 | Transmission Electron Microscopy

Figures 3 and 4 show the average particle size and micrographs of NP, NNP, NC, and NNC. Figure 3 displays the average particle size and TEM images of NP and NNP, whereas Figure 4 displays NC and NNC. ImageJ software was used to modify the scale and threshold to increase the contrast between the particles and the backdrop. Based on the enlarged pictures, it was found that the average particle sizes (diameters) of NP, NNP, NC, and NNC were 4.00 $\pm$ 0.52 nm, 7.00 $\pm$ 0.64 nm, 5.24 $\pm$ 0.28 nm, and 7.92 $\pm$ 0.88 nm, respectively.

TABLE 1 | Constituents of the *Psidium guajava* essential oil.

S/No	Retention time (mins)	Constituent	Retention index	Occurrence (%)	Molecular weight (g/mol)
1.	4.205	α-thujene	925	0.92	136.23
2.	5.135	α-pinene	944	0.68	136.24
3.	5.495	Myrcene	981	1.01	136.23
4.	9.851	β-caryophyllene	982	22.44	204.36
5.	10.750	Limonene	1034	9.24	136.24
6.	11.157	Eucalyptol	1054	0.94	154.25
7.	11.801	Linalool	1121	0.24	154.25
8.	12.331	Eugenol	1389	1.84	164.2
9.	12.366	Aromadendrene	1429	0.28	288.25
10.	12.421	α-humulene	1454	4.76	204.36
11.	12.661	γ-muurolene	1464	1.24	204.35
12.	12.772	Globulol	1576	0.62	222.37
13.	12.931	Caryophyllene oxide	1588	2.46	220.35
14.	13.002	Guaiol	1642	0.42	222.37

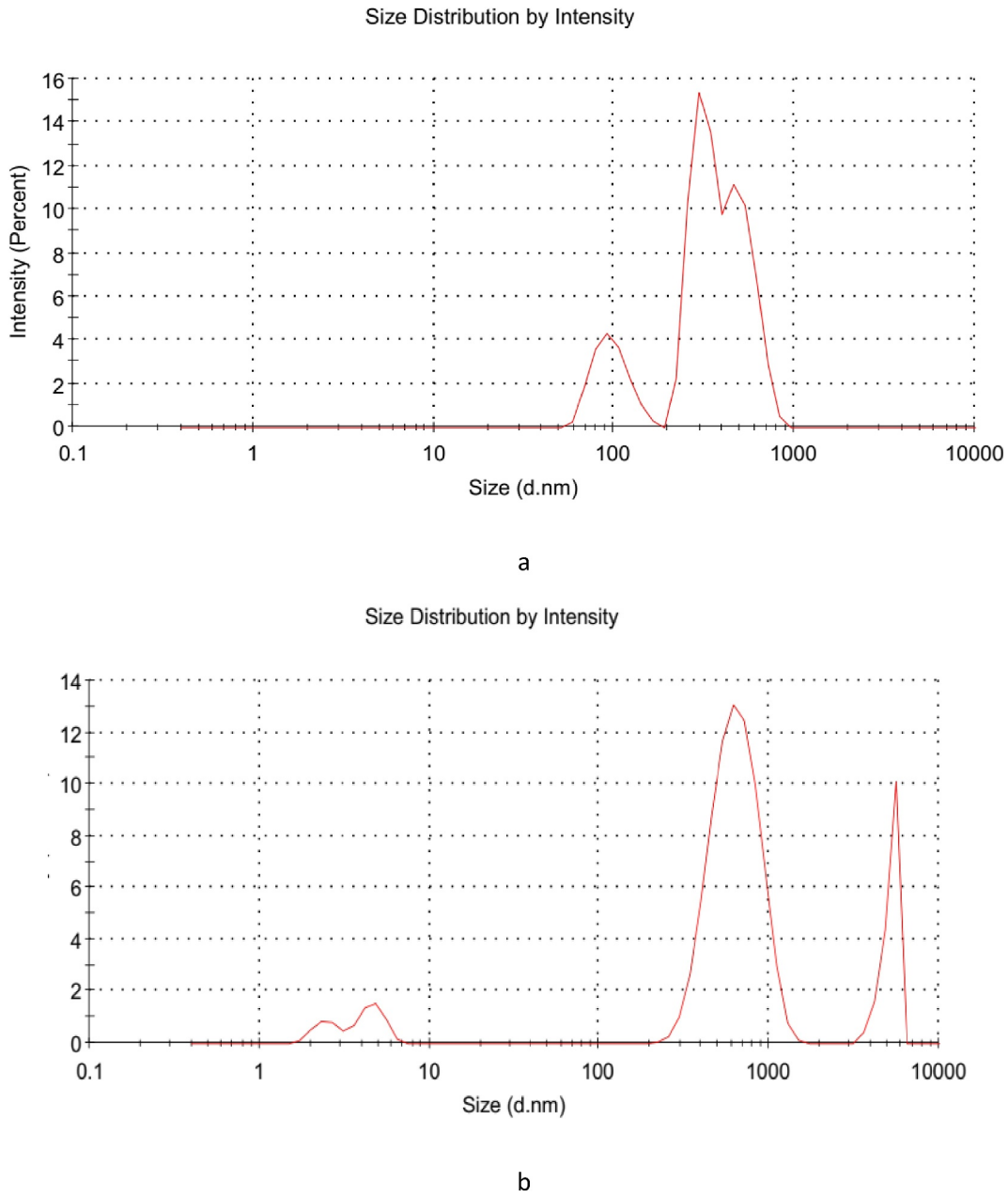


FIGURE 1 | (a) NNP: chitosan nanoencapsulation via solvent displacement method and (b) NP: chitosan nanoencapsulation with guava oil via solvent displacement method.

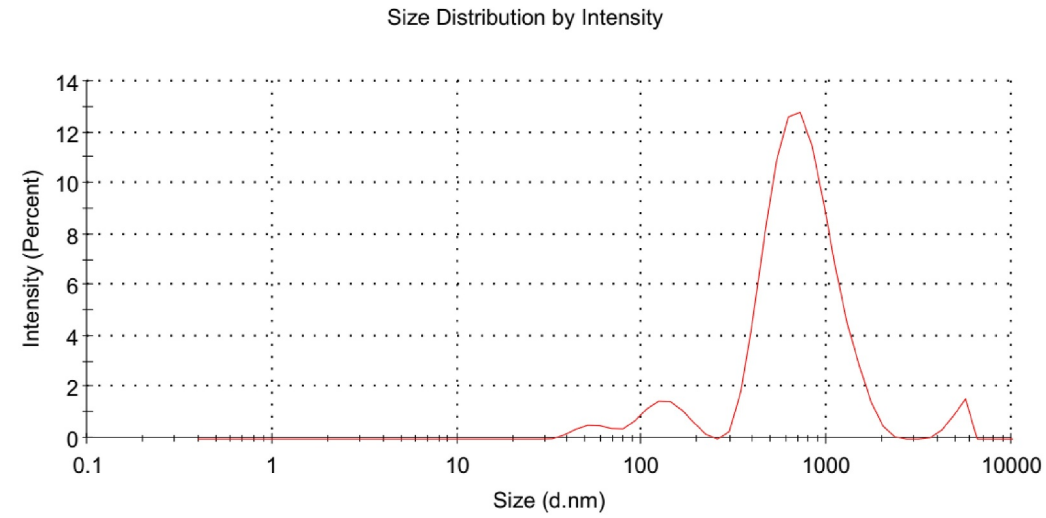
3.2.3 | Loading Capacity and Encapsulation Efficiency

Table 3 shows the loading capacity and encapsulation efficiency of chitosan nanoencapsule (NNP) and chitosan encapsulation with guava oil (NP) via solvent displacement and chitosan (NNC) and chitosan encapsulation with guava oil (NC) via nanoelaboration. As illustrated in the table, incorporating the oil phase into the nanocapsules via the solvent dispersion (NP) or nanoelaboration (NC) methods led to increased loading capacity although at the same time decreasing the encapsulation efficiency (NP: 8.43%, NNP: 9.56%, NC: 10.32%, and NNC: 12.26%).

The increased loading capacity of the nanoencapsulated chitosan upon adding the oil phase was observed, with the values increasing from 8.23% for NNP to 10.23% for NP and from 4.1% for NNC to 6.27% for NC.

3.2.4 | In Vitro Release Kinetics

The analysis of the release kinetics data indicated that the zero-order, first-order, and Higuchi models (R^2) values ranging from 0.9381 to 0.9941 exhibited good fit based on the coefficients of determination (R^2). The rate constants (K_0) for



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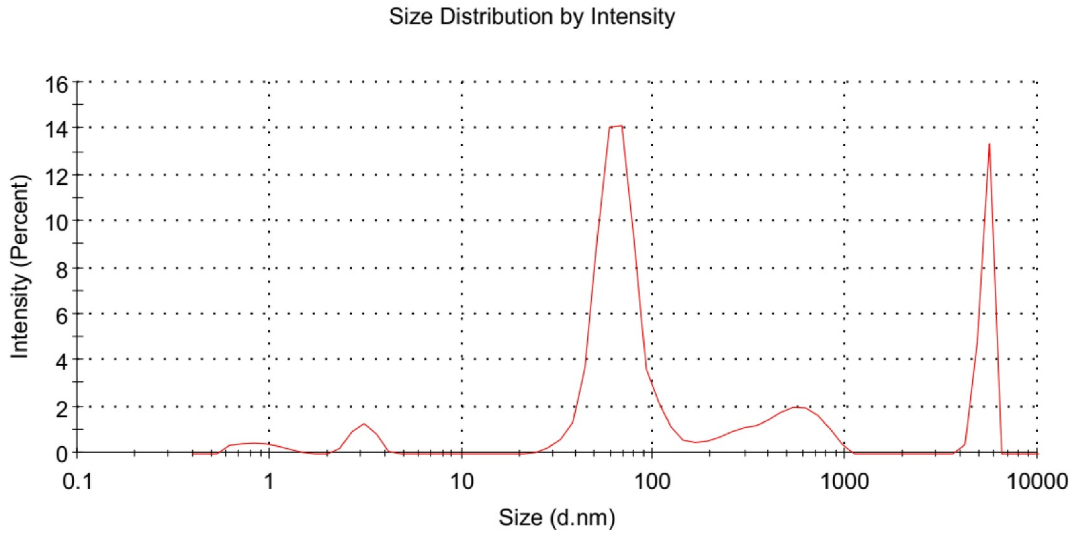

b

FIGURE 2 | (a) NNC: chitosan nanoencapsulation via nanoelaboration method and (b) NC: chitosan nanoencapsulation with guava oil via nanoelaboration method.

TABLE 2 | Zeta potential and particle size (*Z*-average) of the nanocapsules.

S/No		Zeta potential ± SD (mV)	Particle size ± SD (*Z*-average, nm)
1.	NNP	27.1 ± 3.71	443.5 ± 57.72
2.	NP	7.34 ± 5.75	1064 ± 30.52
3.	NNC	50.2 ± 6.83 mV	576.9 ± 316.6
4.	NC	58.8 ± 7.47 mV	1687 ± 20.68

Abbreviations: NC, chitosan nanoprecipitation containing guava oil via nanoelaboration method; NNC, chitosan nanoprecipitation via nanoelaboration method; NNP, chitosan nanoprecipitation via solvent displacement; NP, chitosan nanoprecipitation containing guava oil via solvent displacement.

NNC and NC and NNP and NP were 0.0019 ± 0.014 and 0.0037 ± 0.019 and 0.0013 ± 0.011 and 0.0026 ± 0.018, respectively. Additionally, the rate constants (K_1) for the formation of NNC, NC, NNP, and NP were 0.0691 ± 0.012 and 0.0731 ± 0.014 and 0.072 ± 0.028 and 0.076 ± 0.14, respectively. Furthermore, the rate constants (K_H) for NNC and NC and NNP and NP were 0.004 ± 0.09 and 0.006 ± 0.212 and 0.003 ± 0.11 and 0.005 ± 0.14, respectively.

3.3 | Fungi Isolated From Infected *C. frutescens* Fruits

Figure 5 shows the following fungal species as isolated fungi from *C. frutescens* fruits: *Rhizopus stolonifer*, *Colletotrichum gloeosporioides*, and *Aspergillus niger*.

3.3.1 | Pathogenicity Test

The pathogenicity test of these fungi on inoculated healthy fruits revealed that the majority of the isolated organisms exhibited symptoms and cultural characteristics consistent with *Rhizopus stolonifer* and *Colletotrichum gloeosporioides* (Figure 6a-f).

3.3.2 | In Vitro Antifungal Activity

Figure 7 depicts the in vitro effects of NNP, NP, NNC, and NC after 10 days on *C. gloeosporioides* and *R. stolonifer*. This graph illustrates that there were substantial variations between the examined samples. In comparison to the noninhibitory impact of the control samples of *C. gloeosporioides* (Day 5: 2.32 ± 0.02 cm and Day 10: 4.4 ± 0.04 cm) and *R. stolonifer* (Day 5: 3.95 ± 0.05 cm and Day 10: 5.58 ± 0.07 cm) to NNC (*C. gloeosporioides*-Day 5: 0.57 ± 0.16 cm and Day 10: 3.22 ± 0.09 cm and *R. stolonifer*-Day 5: 2.66 ± 0.09 cm and Day 10: 3.94 ± 0.04 cm) and NNP (*C. gloeosporioides*-Day 5: 1.05 ± 0.14 cm and Day 10: 2.88 ± 0.06 cm and *R. stolonifer*-Day 5: 0.1 ± 0.21 cm and Day 10: 2.47 ± 0.69 cm) that exhibited comparable inhibitory activity. NP was also the most active of the test samples against *C. gloeosporioides* (Day 5: 0.000 cm and Day 10: 0.033 ± 0.08 cm) and *R. stolonifer* (Day 5: 0.000 cm, Day 10: 0.08 ± 0.08 cm), followed by NC (*C. gloeosporioides*-Day 5: 0.81 ± 0.41 cm and Day 10: 1.76 ± 0.04 cm) and *R. stolonifer* (Day 5: 0.58 ± 0.20 cm and Day 10: 1.39 ± 0.04 cm).

TABLE 3 | Oil loading and encapsulation efficiency (E. E) for the nanocapsules.

Sample	Oil loading (%)	Encapsulation efficiency (%)
NNC	4.1	12.26
NC	6.27	10.32
NNP	8.23	9.56
NP	10.23	8.43

3.3.3 | In Vivo Antifungal Activity

The in vivo examination of NP, NNP, NC, and NNC on *C. gloeosporioides* and *Rhizopus stolonifer* demonstrated that NP and NC reduced the incidence and severity of chili pepper disease substantially (Table 4).

3.4 | Quality Assessment of the Effect of Chitosan Nanoprecipitation Containing Guava Oil on Chili Pepper

Figure 8 displays the visual condition of the treated fruits during 28 days of storage at room temperature. The control

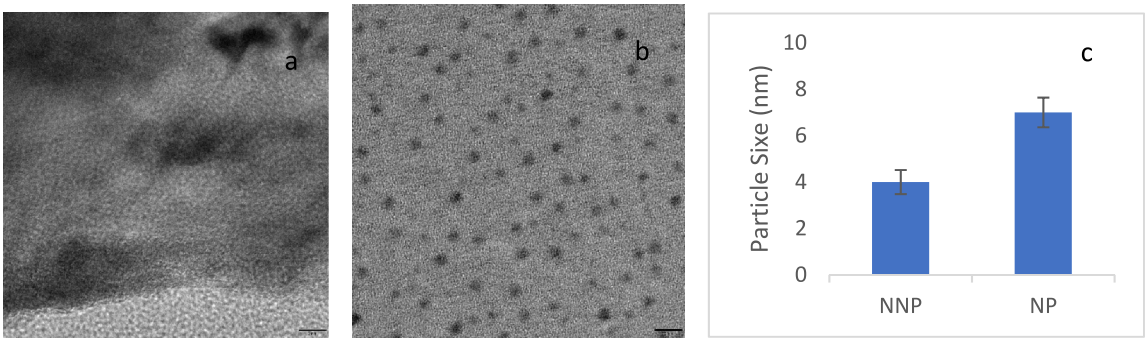

FIGURE 3 | (a) Average particle size and micrographs of (b) NP and (c) NNP.

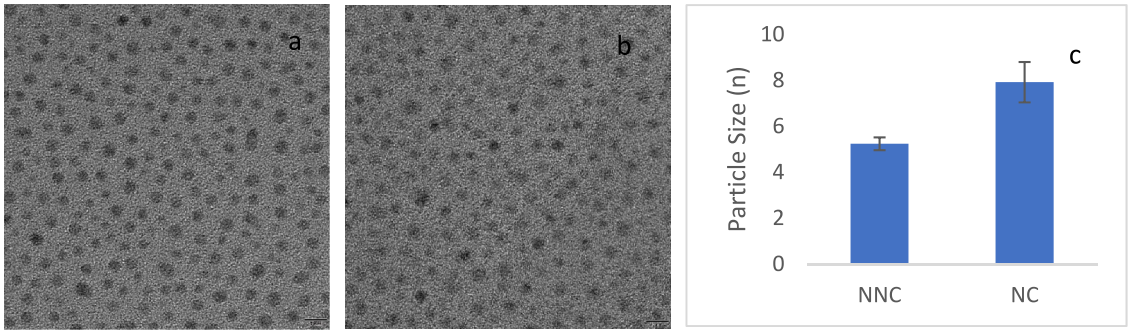

FIGURE 4 | (a) Average particle size and micrographs of (b) NC and (c) NNC.

TABLE 4 | In vivo assessment of NP, NNP, NC, and NNP on *Colletotrichum gloeosporioides and Rhizopus stolonifer*.

Test sample	Incidence (%)	Severity (%)
NP	7.9[a]	5.4[a]
NNP	46.8[b]	41.2[b]
NC	11.6[c]	17.4[c]
NNC	34.4[b]	46.5[b]
CONTROL	69.3[d]	76.4[d]

[a]NP: Chitosan Nanoprecipitation containing guava oil via Solvent Displacement Method.
[b]NNP: Chitosan Nanoprecipitation via Solvent Displacement method; NNC: Chitosan Nanoprecipitation via Nanoelaboration Method.
[c]NC: Chitosan Nanoprecipitation containing guava oil via nanoelaboration method.
[d]Control: Untreated sample.

treatment (CTRL), followed by NNP and NNC, demonstrated more notable effects on fruit appearance on day 28.

3.4.1 | Weight Loss and Water Activity

Chitosan nanoprecipitation containing guava oil through solvent displacement method (NP) and nanoelaboration (NC) methods, as well as chitosan nanoprecipitate without oil through solvent dispersion (NNP) and nanoelaboration (NNC) methods, significantly influenced weight loss and water activity for the chilli pepper fruits ($p \leq 0.05$). Figure 9 depicts the increase in weight loss during storage of chili peppers treated with nanoprecipitation materials. Without treatment, there was a greater increase in weight loss (CTRL, Day 7:

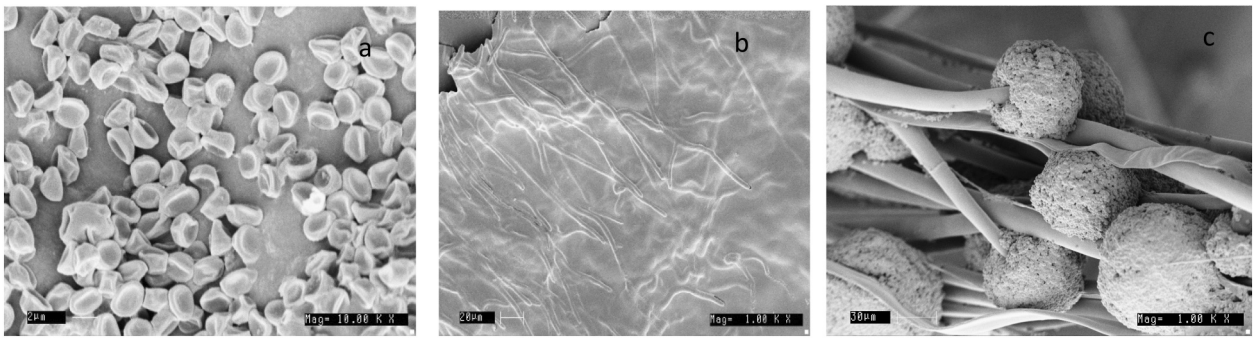

FIGURE 5 | (a–c) SEM micrographs of (a) *Rhizopus stolonifer*, (b) *Colletotrichum gloeosporioides*, and (c) *Aspergillus niger*.

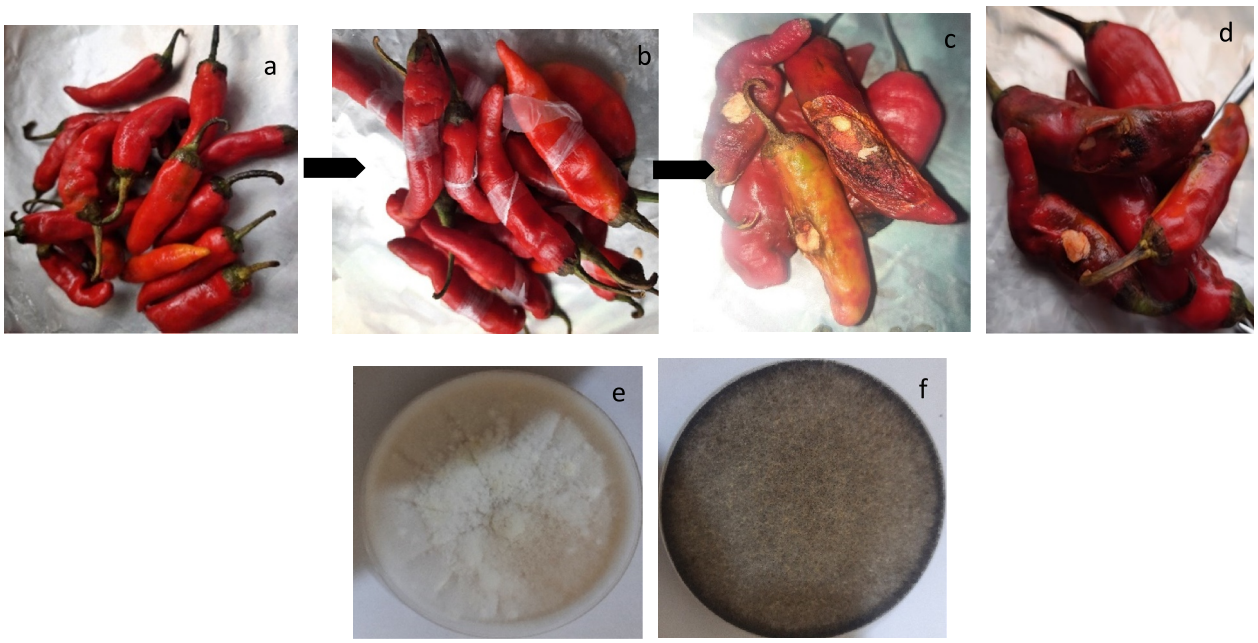

FIGURE 6 | (a) Chili peppers before inoculation for pathogenicity test, (b) innoculated chili peppers, (c and d) diseased chili peppers that was used as reisolated fruits for pathogenicity, (e) reisolated *Colletotrichum gloeosporioides* after pathogenicity test, and (f) reisolated *Rhizopus stolonifer* after pathogenicity test.

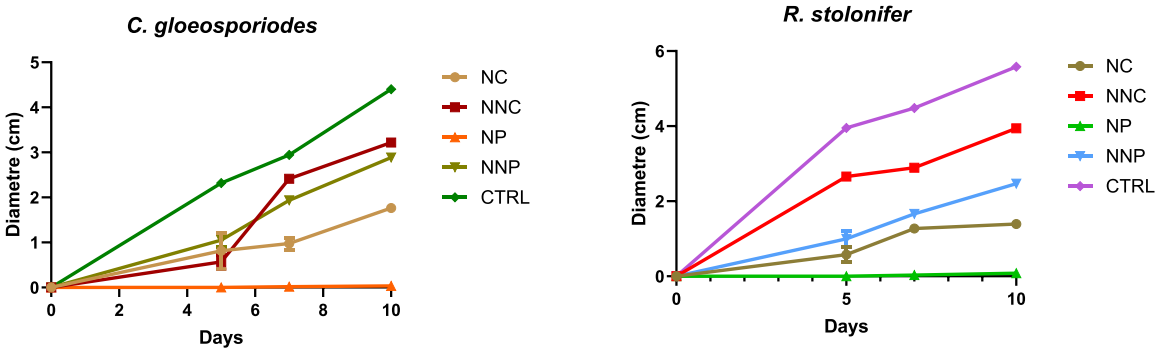

FIGURE 7 | In vitro activities of NNP, NP, NNC, and NC on *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. NC, chitosan nanoprecipitation containing guava oil via nanoelaboration method; NNC, chitosan nanoprecipitation via nanoelaboration method; NNP, chitosan nanoprecipitation via solvent displacement; NP, chitosan nanoprecipitation containing guava oil via solvent displacement.

TABLE 5 | LC_{50} of chitosan nanoprecipitation containing essential oil and with essential oil.

S/No	Treatment	LC_{50} (μg/mL)	Regression equation	R^2 (%)	*p*-values	Chi-squared	Inference
1.	NNP	3071.72	$Y = 0.263X \pm 0.917$	91.9	0.32	0.500	Nontoxic
2.	NP	2089.87	$Y = 0.315X \pm 1.047$	95.2	0.013	0.341	Nontoxic
3.	NNC	5512.64	$Y = 0.242X \pm 0.907$	85.2	0.048	0.788	Nontoxic
4.	NC	1314.86	$Y = 0.2734X \pm 0.851$	88.1	0.025	0.667	Nontoxic
5.	Control	7389.61	$Y = 0.288X \pm 1.115$	92.4	0.025	0.478	Nontoxic
6.	$K_2Cr_2O_7$	66.88	$Y = 0.367X \pm 0.679$	97.1	0.002	0.239	Toxic

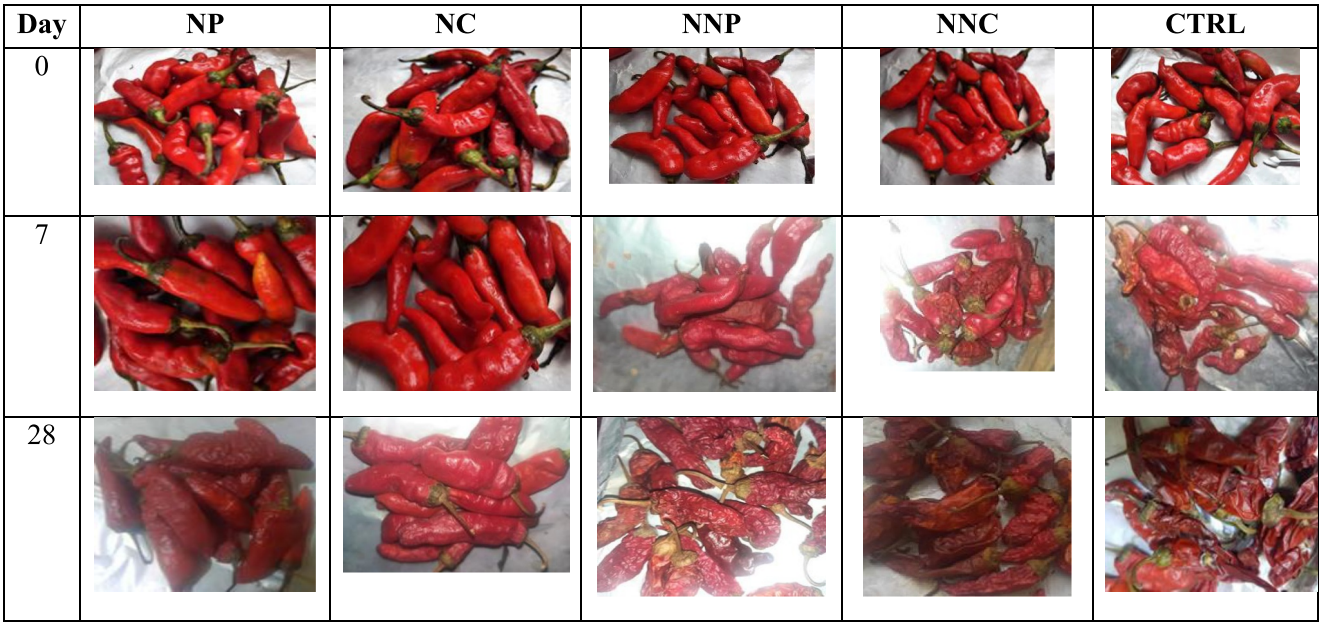

FIGURE 8 | Visual assessment of the treated chili pepper stored for 28 days.

8.24 ± 0.63% and Day 28: 31.05 ± 0.96%) than with treatment (NC, Day 7: 4.48 ± 0.18% and Day 28: 9.76 ± 0.11%; NP, Day 7: 3.4 ± 0.19% and Day 28: 9.60 ± 0.25%; NNC, Day 7: 5.99 ± 0.49% and Day 28: 17.95 ± 0.38%; and NNP, Day 7: 6.47 ± 0.62% and Day 28: 21.16 ± 1.12%).

The increase in water activity treated with nanoprecipitation materials during storage is seen in Figure 10. Without treatment (CTRL, Day 7: 0.92 ± 0.01 and Day 28:0.99 ± 0.06), water activity increased more than with the treatments (NC, Day 7: 0.74 ± 0.01 and Day 28: 0.85 ± 0.01; NP, Day 7: 0.72 ± 0.01 and Day 28:

0.82 ± 0.05; NNC, Day 7: 0.83 ± 0.01 and Day 28: 0.95 ± 0.03; and NNP, Day 7: 0.87 ± 0.01 and Day 28: 0.93 ± 0.06).

3.4.2 | Total Soluble Solid and Firmness

Chitosan nanoprecipitation containing guava oil by solvent displacement method (NP) and nanoelaboration (NC), as well as chitosan nanoprecipitation without oil via solvent displacement method (NNP) and nanoelaboration (NNC) methods, significantly contribute to the increase in total soluble solid and reduction in the firmness in chili pepper fruits ($p \leq 0.05$). The increase in total soluble solid during storage of chili peppers treated with nanoprecipitation materials is illustrated in Figure 11. The larger rise in CTRL (Day 7: 5.9°Brix and Day 28: 7.6°Brix) in comparison to the low increase in treated samples (NC, Day 7: 4.8°Brix and Day 28: 5.3°Brix; NP, Day 7: 4.7°Brix and Day 28: 5.2°Brix; NNC, Day 7: 5.8°Brix and Day 28: 7.00°Brix; and NNP, Day 7: 5.8°Brix and Day 28: 6.9°Brix).

Figure 12 demonstrates the decrease in firmness following the use of the nanoprecipitation material for the storage of chili pepper. Without treatment (CTRL, Day 7: 26.70 ± 0.63 N and Day 28: 0.66 ± 0.12 N), firmness decreased significantly compared to the treated samples (NC, Day 7: 48.85 ± 0.41 N and Day 28: 28.34 ± 0.55 N; NP, Day 7: 53.90 ± 2.00 N and Day 28: 30.95 ± 0.27 N; NNC, Day 7: 24.76 ± 0.05 N and Day 28: 2.35 ± 0.57 N; and NNP, Day 7: 30.49 ± 0.57 N and Day 28: 1.73 ± 0.41).

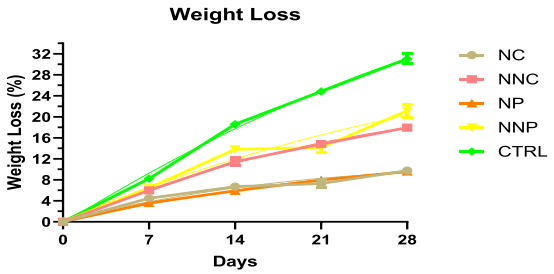

FIGURE 9 | Weight loss. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil. NC, chitosan nanoprecipitation integrated with guava oil via nanoelaboration method; NNC, chitosan nanoprecipitation via nanoelaboration method; NNP, chitosan nanoprecipitation via solvent displacement; NP, chitosan nanoprecipitation integrated with guava oil via solvent displacement.

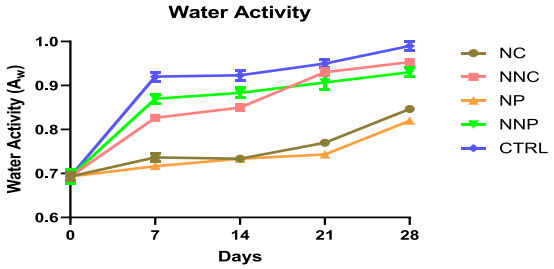

FIGURE 10 | Water activity. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil. NC, chitosan nanoprecipitation integrated with guava oil via nanoelaboration method; NNC, chitosan nanoprecipitation via nanoelaboration method; NNP: chitosan nanoprecipitation via solvent displacement; NP, chitosan nanoprecipitation integrated with guava oil via solvent displacement.

3.4.3 | pH and Electrolyte Leakage

Chitosan nanoprecipitation containing guava oil via solvent displacement method (NP) and nanoelaboration (NC) methods and chitosan nanocapsules without oil via solvent displacement (NNP) and nanoelaboration (NNC) methods both significantly improve the quality of chili pepper fruits by delaying the continuous activities of pH and electrolyte leakage in chili pepper fruits ($p \leq 0.05$) during 28-day storage. Figure 13 depicts how untreated sample (CTRL: Day 7: 5.37 ± 0.04 and Day 28: 3.70 ± 0.09) decreased more than the treated samples (NC, Day 7: 5.37 ± 0.05 and Day 28: 4.83 ± 0.02; NP, Day 7: 5.35 ± 0.05 and Day 28: 4.93 ± 0.04; NNC, Day 7: 5.32 ± 0.07 and Day 28: 4.05 ± 0.02; NNP, Day 7: 5.23 ± 0.03 and Day 28: 4.03 ± 0.03).

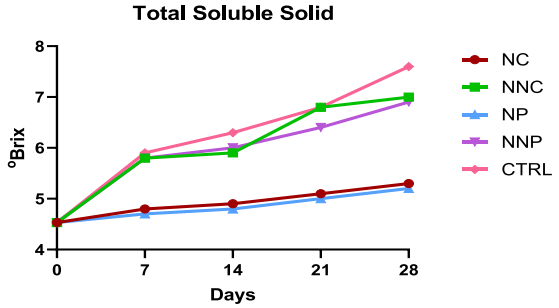

FIGURE 11 | Total soluble solid. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil.

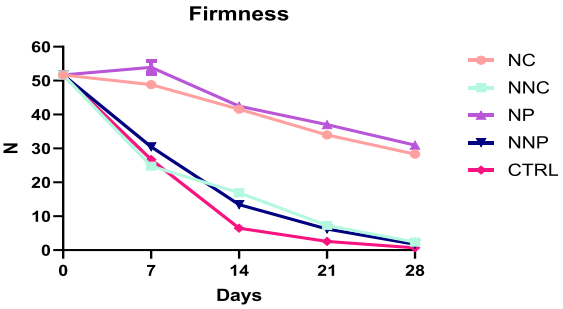

FIGURE 12 | Firmness. Chitosan nanoprecipitation with oil and chitosan nanoprecipitation without oil.

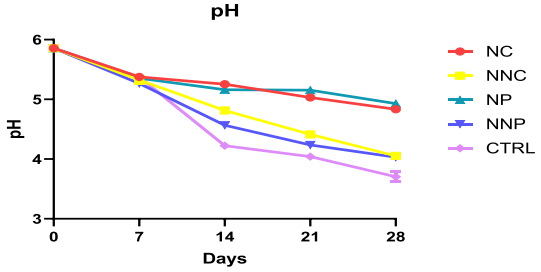

FIGURE 13 | pH. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil.

Figure 14 depicts the electrolyte leakage that happened when the chili pepper was coated with nanoencapsulation material. The untreated samples (CTRL: Day 7: 3.57 ± 0.19 and Day 28: 7.81 ± 0.09) were significantly softer than the treated (NC, Day 7: 3.57 ± 0.18 and Day 28: 4.75 ± 0.03; NP, Day 7: 3.23 ± 0.01 and Day 28: 4.2 ± 0.02; NNC, Day 7: 3.56 ± 0.17 and Day 28: 7.86 ± 0.031; NNP, Day 7: 3.57 ± 0.07 and Day 28: 7.46 ± 0.13).

3.4.4 | Antioxidant Capacity, Browning Index, and Total Phenolic Content

The effects of chitosan nanoprecipitation containing guava oil on the antioxidant capacity and browning index of chili pepper fruit after 28 days of storage are depicted in Figures 15 and 16. Compared to antioxidant capacity of untreated chili pepper (CTRL: Day 7: 53.67 ± 1.20% and Day 28: 43 ± 0.58%) and browning index of untreated chili pepper (CTRL: Day 7: 0.03 ± 0.001% and Day 28: 0.13 ± 0.002%), the effect of the coating treatment on antioxidant capacity of chili pepper (NC, Day 7: 57 ± 0.58% and Day 28: 58.33 ± 0.33%; NP, Day 7: 57.33 ± 0.88% and Day 28: 60.67 ± 0.88%; NNC, Day 7: 56.33 ± 1.20% and Day 28: 47.33 ± 0.67%; NNP, Day 7: 56 ± 0.58% and Day 28: 52.33 ± 0.88%) and the browning index of chili pepper (NC, Day 7: 0.029 ± 0.002 and Day 28: 0.051 ± 0.001; NP, Day 7: 0.028 ± 0.00 and Day 28: 0.05 ± 0.002; NNC, Day 7: 0.03 ± 0.001 and Day 28: 0.11 ± 0.003; NNP, Day 7: 0.03 ± 0.001 and Day 28: 0.11 ± 0.01) showed a greater influence on the freshness of the chili pepper fruits during storage for 28 days.

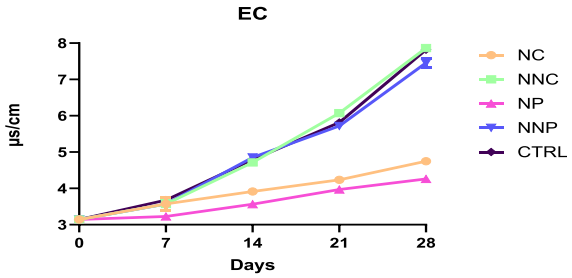

FIGURE 14 | Electrolyte leakage. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil.

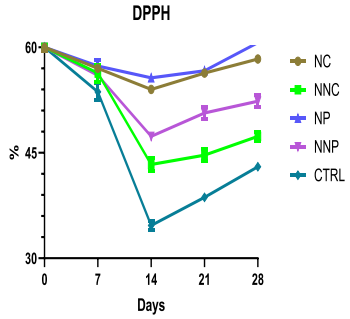

FIGURE 15 | Antioxidant capacity. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil.

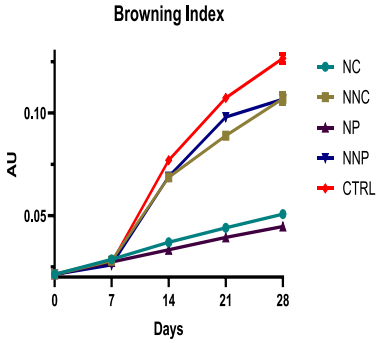

FIGURE 16 | Browning index. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil.

According to the nonlinear regression curves in Figure 17, the time of storage had an effect on the rise in the total phenolic content of all test samples. Chilli pepper fruits stored for 28 days without treatment (CTRL: Day 7: 25.23 ± 0.4GAE mg 100 g^{-1} and Day 28: 9.6 ± 0.314GAE mg 100 g^{-1}) had lower total phenolic content during storage for 28 days ($p \leq 0.05$). The addition of the coating on the chili pepper fruits (NC, Day 7: 25.13 ± 0.93 GAE mg 100 g^{-1} and Day 28: 29.26 ± 1.04 GAE mg 100 g^{-1}; NP, Day 7: 24.66 ± 0.30 GAE mg 100 g^{-1} and Day 28: 28.71 ± 0.29 GAE mg 100 g^{-1}; NNC, Day 7: 23.53 ± 0.33 GAE mg 100 g^{-1} and Day 28: 16.40 ± 0.61 GAE mg 100 g^{-1}; NNP, Day 7: 26.22 ± 0.74 GAE mg 100 g^{-1} and Day 28: 17.82 ± 0.52 GAE mg 100 g^{-1}) influenced the lower total phenolic content of the chili pepper fruits during storage for 28 days.

3.5 | Effect of Chitosan Nanoprecipitation Containing Guava Oil on Activity of Defense Related Enzymes During Storage

To further explain the inhibitory effect of the chitosan nanoprecipitation containing guava oil and chitosan nanoprecipitate on the disease in chili pepper, the coating process contributes to an increase in polyphenol oxidase (PPO) in chili pepper as illustrated in Figure 18. The untreated inoculated chili pepper demonstrated a significant increase in PPO activity (Day 7: 0.29 ± 0.00 U/g and Day 28: 1.07 ± 0.02 U/g) compared to the treated inoculated fruits, including NP (Day 7: 0.22 ± 0.09 U/g and Day 28: 0.46 ± 0.07 U/g), NC (Day 7:

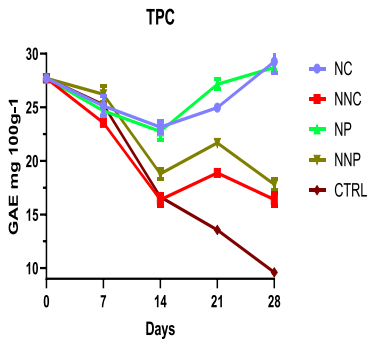

FIGURE 17 | Total phenolic content ss. Chitosan nanoprecipitation with oil and chitosan nanoprecipitate without oil.

0.23 ± 0.01 U/g and Day 28: 0.45 ± 0.04 U/g), NNC (Day 7: 0.26 ± 0.05 U/g and Day 28: 0.45 ± 0.01 U/g), and NNP (Day 7: 0.28 ± 0.06 U/g and Day 28: 0.45 ± 0.04 U/g) during the 28-day storage period.

The pattern of β-1,3-glucanase activity in Figure 19 showed an opposite trend to that of polyphenol oxidase (PPO) activity (Figure 18). Among the fruits inoculated with *C. gloeosporioides*, the highest level of β-1,3-glucanase activity was recorded in chili peppers treated with NP (Day 7: 3.33 ± 0.33 U/g and Day 28: 7.6 ± 0.28 U/g), followed by NC (Day 7: 3.67 ± 0.48 U/g and Day 28: 7.3 ± 0.33 U/g), NNC (Day 7: 3.33 ± 0.62 U/g and Day 28: 4.3 ± 0.46 U/g), and NNP (Day 7: 3.33 ± 0.24 U/g and Day 28: 4.67 ± 0.38 U/g), whereas the untreated fruits showed the lowest level of activity (CTRL, Day 7: 3.67 ± 0.43 U/g and Day 28: 2.3 ± 0.24 U/g) of β-1,3-glucanase activity.

Figure 20 illustrates the changes in phenylalanine ammonia-lyase (PAL) activity of chili pepper over a 28-day storage period after inoculated fruits were treated with chitosan nanoprecipitation with guava oil, chitosan nanoprecipitate, and control treatments. PAL activity increased significantly from Day 0 to Day 14 in all treated samples, followed by a gradual decrease from Day 14 to Day 28. On the other hand, there was no significant difference in PAL activity in the untreated sample from Day 7 to Day 14 and it decreased from Day 21 to Day 28. The study found that chitosan nanoprecipitation with guava oil

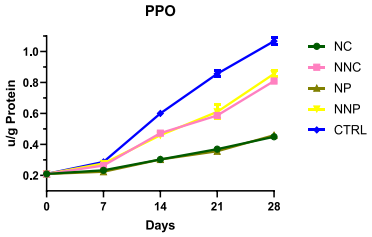

FIGURE 18 | Polyphenol oxidase.

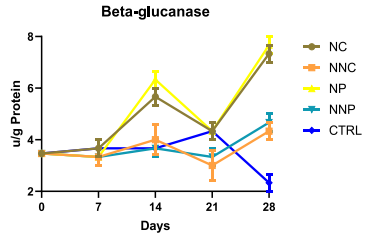

FIGURE 19 | β, 1-3-glucanase.

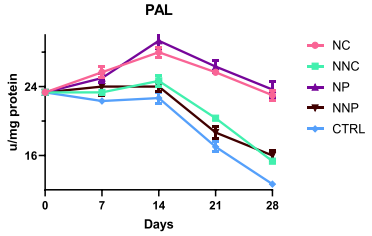

FIGURE 20 | Phenylalanine ammonia lyase.

treatment (NP) via solvent dispersion method resulted in the highest PAL activity during the 28-day storage period, with values of 25.00 ± 0.56 U/g on Day 7, 29.33 ± 0.67 U/g on Day 21, and 23.67 ± 0.88 U/g on Day 28. The PAL activity of the chili pepper treated with chitosan nanoprecipitation with guava oil treatment (NC) via the nanoelaboration method was slightly lower than NP, with values of 25.67 ± 0.67 U/g on Day 7, 25.67 ± 0.33 U/g on Day 21, and 23.00 ± 0.58 U/g on Day 28. The PAL activity of the chili pepper treated with chitosan nanoprecipitation (NNP) via solvent displacement method was lower than NP and NC, with values of 24.00 ± 1.0 U/g on Day 7, 18.67 ± 0.67 U/g on Day 21, and 16.00 ± 0.58 U/g on Day 28. Finally, the untreated inoculated chili pepper had the lowest PAL activity during the 28-day storage period, with values of 22.33 ± 0.33 U/g on Day 7, 17.00 ± 0.58 U/g on Day 21, and 12.67 ± 0.33 U/g on Day 28.

3.6 | Effect of Chitosan Nanoprecipitation Containing Guava Oil on *Artemia salina*

Table 5 depicts the lethal concentration that killed 50% of the population of brine shrimp assessed with chitosan nanoprecipitation containing guava oil via the solvent displacement method (NP) and nanoelaboration (NC) method as well as chitosan nanocapsules without oil via solvent displacement (NNP) and nanoelaboration (NNC) methods. The table shows that NNP has an LC_{50} of 3071.72 μg/mL, NP has an LC_{50} of 2089.86 μg/mL, NNC has an LC_{50} of 5512.64 μg/mL, NC has an LC_{50} of 1314.86 μg/mL, the control treatment (seawater) has an LC_{50} of 7389.61 μg/mL, and potassium dichromate ($K_2Cr_2O_7$) has an LC*50* of 66.88 μg/mL.

4 | Discussion

This study investigates the use of chitosan nanoprecipitation containing guava oil to address the deterioration of chili pepper fruits caused by disease-causing pathogens. It focuses on controlling disease-causing fungi, improving the shelf life, and analyzing the influence on antioxidant enzymes. In furtherance of establishing the effect of chitosan nanoprecipitation containing essential oil, the phytochemical components of *Psidium guajava* essential oil, as determined via GCMS and depicted in Table 1, revealed that β-caryophyllene constitutes the highest composition. In contrast, limonene and α-humulene exhibit considerable compositions following β-caryophyllene. Notably, studies by Alam et al. (2023), El-Ahmady et al. (2013), and Lee et al. (2011) have documented comparable β-caryophyllene contents, specifically 27.98%, 16.90%, and 6.80%, respectively, compared to the 22.44% reported in this study. In addition, the limomene content (9.24%) reported in this study is lower than what has been reported by Chaturvedi et al. (2021), Soliman et al. (2016), and Zaka et al. (2015), where concentrations of 29.1%, 54.7%, and 53.80% were recorded, respectively. Moreover, the findings of Khadhri et al. (2014) indicated a higher α-humulene content of 10.93%, contrasting with the lower concentration of 4.76% established in this study.

This study further established that the distribution of particle sizes varied among the various preparation methods and formulations. The Z-average of chitosan nanoencapsulate (NNP) prepared through solvent displacement nanoprecipitation was 443.5 nm, whereas the Z-average of chitosan nanoencapsulated guava oil (NP) prepared using the same method was 1064 nm. Conversely, the Z-averages of chitosan nanoencapsulate (NNC) and chitosan nanoencapsulated guava oil (NC) prepared using the nanoelaboration method were 576.9 and 1687 nm, respectively. The substantial increase in particle size that occurs upon oil loading indicates the effective integration of guava oil into the chitosan matrix. This incorporation can be attributed to the self-association of the hydrophobic segments of guava essential oil within and around the chitosan structure, which forms a cage-like arrangement with the hydrophilic segments (F. Zhang et al. 2020).

In addition, a high magnitude of either positive or negative zeta potential is essential to prevent particle aggregation. High zeta potential enhances repulsive forces, thereby overcoming attractive forces and preventing agglomeration (L. M. Ali et al. 2019). Unlike the chitosan nanoparticles synthesized with guava leaf extract established by L. M. Ali et al. (2019), which showed negative zeta potentials on the surface of particles, this study established that the chitosan nanoparticles exhibited positive zeta potentials: NNP: 27.1 $\pm$ 3.71 mV, NP: 7.34 $\pm$ 5.75 mV, NNC: 50.2 $\pm$ 6.83 mV, and NC: 58.8 $\pm$ 7.47 mV. The positive zeta potentials are attributed to the cationic nature of chitosan. Interestingly, the effect of oil loading on zeta potential varied between methods. For solvent displacement, oil loading decreased the zeta potential, whereas for nanoelaboration, it increased. This difference suggests that the preparation method significantly influences the surface characteristics of the nanoparticles. Based on the study by Benamer Oudih et al. (2023), zeta potentials more positive than +30 mV or more negative than −30 mV are typically considered stable. Hence, this explains the greater stability of NC.

Moreover, this study established that the TEM analysis (NP: 4.00 $\pm$ 0.52 nm, NNP: 7.00 $\pm$ 0.64 nm, NC: 5.24 $\pm$ 0.28 nm, and NNC: 7.92 $\pm$ 0.88 nm) revealed much smaller particle sizes than DLS measurements. This discrepancy between DLS and TEM measurements is consistent with what was reported by Matche and Adeogun (2022) for nanoencapsulated *Eucalyptus globulus* oil with gum Arabic. It can be attributed to similar factors, such as the dry state of TEM samples and the measurement of hydrodynamic radius in DLS versus physical size in TEM. Our results corroborate the earlier findings of Karimirad et al. (2020), who similarly observed positively charged particle surfaces. They attributed this phenomenon to an elevated mass ratio of essential oil relative to chitosan nanoparticles.

This study also established that the addition of guava oil increased the loading capacity but decreased the encapsulation efficiency (NNP: 8.23% loading and 9.56% efficiency, NP: 10.23% loading and 8.43% efficiency, NNC: 4.1% loading and 12.26% efficiency, and NC: 6.27% loading and 10.32% efficiency). This trend is similar to what Matche and Adeogun (2022) described with nanoencapsulated *Eucalyptus globulus* oil incorporated with gum Arabic study. As Matche and Adeogun (2022) posited, this may be related to the saturation of oil-binding sites in the chitosan matrix. In addition, Rajkumar et al. (2020) established that the increase in oil loading compared to encapsulation efficiency suggested that nanoparticles increased when the initial content of active substance increased, while further increased concentration reduced encapsulation efficiency. Previous research by Soleimani, Mostowfizadeh-Ghalamfarsa, Ghanadian, et al. (2024) also stated that the increase in encapsulation efficiency depended on the composition of wall material, stability, and physicochemical properties of the emulsions.

Furthermore, the release kinetics of guava oil from nanocapsules (NC) and nanoparticles (NP) can be attributed to the encapsulation process and subsequent diffusion across the capsule membrane. The kinetic release of NP and NC closely fits zero-order, first-order, and Higuchi kinetic models as Matche and Adeogun (2022) observed. This conformity with multiple kinetic models suggests that the release mechanism is primarily governed by controlled diffusion through the chitosan nanocapsule membrane, resulting in the sustained release of guava oil and its bioactive compounds. Additionally, previous work by Nunes et al. (2024) corroborated the patterns of kinetic release of guava oil obtained in this study based on what was established in their research. They further established that the release of essential oil depends on several factors, such as the pH of the medium, ionic strength, wall material, particle size, and the solvent used in the process. The interaction between the wall material and the encapsulated material represents a crucial factor that determines both the encapsulation efficiency and the release into the environment.

The pathogenicity assays conducted in this study provided an understanding of the roles of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in the postharvest deterioration of *Capsicum fruescens* (chili pepper) fruits. The results corroborate existing literature on the pathogenic impact of *C. gloeosporioides* and *R. stolonifer* in disease development in chili peppers. *C. gloeosporioides* has been consistently identified in the literature as a pathogen affecting chili peppers, causing significant postharvest losses due to anthracnose disease (Rony et al. 2024; A. Zhang et al. 2024). Furthermore, this study also confirmed the presence of *R. stolonifer* as a causative pathogenic fungus responsible for postharvest disease in chili peppers. The pathogenicity of *C. gloeosporioides* and *R. stolonifer* on chili pepper fruits and other fruits is well documented in literature, extending beyond chili peppers to a diverse range of fruits. These include tropical and subtropical crops such as mango, papaya, ginger, and sweet orange. These fungi typically cause soft rot diseases characterized by rapid tissue maceration and mycelial growth, often leading to significant postharvest losses in various horticultural commodities (Faramitha et al. 2022; Rony et al. 2024; Syamilah et al. 2022; Kossonou et al. 2014; A. Zhang et al. 2024).

In addition, studies by Das et al. (2021), Liu et al. (2016), Moreira et al. (2022), Soppelsa et al. (2023), Vafania et al. (2019), and X. Zhang et al. (2021) have demonstrated that integrating edible coatings, such as chitosan, xanthan, gelatin, tragacanth gum, gum arabic, and guar gum with essential oils, such as eucalyptus oil, thyme oil, peppermint oil, clove oil, and guava oil, can significantly enhance the nanoencapsulation of these oils. This process helps in maintaining the oils' volatility and boosts their antifungal properties. Specifically, based on the

results obtained from this study, nanoencapsulation with guava oil has been shown to be particularly effective (Barradas and de Holanda e Silva 2021; Pateiro et al. 2021). The enhancement in antifungal activity is due to the nanostructured materials adhering to the fungal surfaces, enabling a controlled release of the active compounds close to the fungal cell membranes or facilitating their direct transport to the target areas (de Carvalho et al. 2021; Debeaufort et al. 2023; Kapustová et al. 2021).

Furthermore, as established in the literature and obtained from the GC–MS analysis in this study, the phytoconstituents of guava oil possess significant antifungal properties attributed to its major components. These constituents' presence might contribute to guava oil's overall efficacy in inhibiting fungal growth. For instance, β-caryophyllene, one of the phytoconstituents in guava oil, has been acknowledged for its antimicrobial properties against microorganisms associated with food materials (Moo et al. 2020). In addition, Alam et al. (2023) equally established that β-caryophyllene having a high percentage composition influenced guava oil's antimicrobial activity. Integrating guava oil with an edible coating to form a nanostructured formulation from materials, such as chitosan, can enhance the antifungal capabilities of the formulation due to the large surface-to-volume ratio by improving its interaction with fungal cell walls, increasing adherence and penetration, thus effectively inhibiting fungal growth. This principle is similar to how chitosan-loaded guava essential oil and nanoencapsulated *Eucalyptus globulus* oil have shown effectiveness against multidrug resistant *Klebsiella pneumoniae* and anthracnose disease of *Syzygium malaccense* fruits, respectively (Matche and Adeogun 2022; F. Zhang et al. 2020).

This study further established the antifungal efficacy of chitosan nanoprecipitation integrated with guava oil on chili pepper. The effectiveness of the chitosan nanoprecipitation integrated with guava oil via solvent displacement (NP) and chitosan nanoprecipitation integrated with guava oil via nanoelaboration (NC) formulations against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* was influenced by the addition of guava oil as evidenced by the antifungal activity of NP and NC when compared to NNP and NC. Grande-Tovar et al. (2018) and Matche and Adeogun (2022) observed similar results, noting the enhanced activities of nanostructured edible coatings, such as chitosan integrated, with essential oils such as guava oil. Grande-Tovar et al. (2018) further highlighted that the integration of essential oils (EOs), such as guava oil, with chitosan allows the oil to be encapsulated within the polymer matrix through emulsification in aqueous film-forming dispersions of the polymer. This process is followed by casting and drying under controlled conditions. Additionally, edible chitosan coatings enriched with EOs have been shown to exhibit significant antimicrobial activity, effectively reducing the growth of pathogenic species on fruit surfaces, while preserving overall fruit quality, nutritional compounds, and consumer acceptance.

This study also demonstrated that the nanostructured materials derived from chitosan integrated with guava oil were more efficacious than synthetic preservatives, such as copper oxychloride, azoxystrobin, and tebuconazole (Giacomini et al. 2023; D. Kumar and Kalita 2017). Although synthetic preservatives have been documented to effectively inhibit the growth of fungal pathogens such as *C. gloeosporioides* and *R. stolonifer*, which cause fruit rot in chili pepper, they often leave residues on produce and may pose risks to human health, the environment, and soil toxicity. Furthermore, pathogens can develop resistance to these chemicals over time, diminishing their effectiveness. These issues underscore the importance of studies, such as ours, which explore the use of natural antimicrobials for controlling pathogens such as *C. gloeosporioides* and *R. stolonifer* (Chávez-Magdaleno et al. 2018; Oussou-Azo et al. 2020; S. Sharma et al. 2021).

In addition, the NP formulation exhibited the highest activity, with the formulations used in this study demonstrating substantial inhibition zones against *C. gloeosporioides*. The two pathogenic fungal species exhibited a range of efficacy. The increased antifungal activity is probably a result of the synergistic effects between the guava essential oil and their biopolymer matrix (chitosan), with β-caryophyllene in guava oil contributing to their efficacy (Rakmai et al. 2018).

The study also established the effect of chitosan nanoencapsulated guava oil on the in vivo assessment of chili pepper. The in vivo examination of nanostructured materials (NP, NNP, NC, and NNC) and untreated samples on *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* demonstrated that NP and NC substantially reduced the incidence and severity of chili pepper disease (Table 4). The study further suggested that guava oil contributed to NP and NC's significantly better antifungal activity than NNP and NNC. The reduced incidence and severity in the NP and NC treatments highlight the effectiveness of guava oil in mitigating fungal infections due to the improvement in the adhesion and diffusion of the oil into the fungal cell walls, thereby restricting fungal development. Although no studies have been conducted on the impact of nanostructured chitosan incorporated with guava oil, similar antifungal activities have been established for pepper tree and *Origanum vulgare* L. essential oils-loaded chitosan bionanocomposites on in vivo postharvest control of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* (Chávez-Magdaleno et al. 2018; dos Santos et al. 2012).

Further evaluation of chitosan nanoprecipitated guava oil's impact on chili pepper quality assessment established its efficacy in extending the chili's shelf life for 28 days. The nanosynthesized incorporations of guava oil with chitosan (NP and NC) significantly reduced weight loss and water activity in chili peppers during storage. This outcome mirrors the efficacy observed with *Heracleum persicum* essential oil integrated with chitosan nanoparticles in reducing weight loss in bell peppers as Taheri et al. (2020) reported. Taheri et al. (2020) posited that the reduced weight loss might be due to the presence of essential oil in the coating, which creates barriers that slow down the fruit's release of water and carbon dioxide, as well as the process of water evaporation from the fruit's surface. This barrier effect helps retain moisture within the peppers, thus minimizing weight loss (Zare-Bavani et al. 2024). In addition, NP and NC showed lower water activity with chili pepper due to the influence of nanostructured chitosan with guava oil, which enhances this effect because of its hydrophobic nature. This occurs because the antimicrobial agents in the coating alter how water and sugar molecules interact to form hydrogen bonds within the

polysaccharide matrix, reducing the availability of hydroxyl groups and enhancing the resistance to water loss in chili pepper (Chaudhary et al. 2020; Qiao et al. 2019).

Chitosan nanoprecipitation incorporated with guava oil (NP and NC) effectively contributed to maintaining a lower increase in the TSS of chili peppers during storage compared to untreated controls. Over a 28-day period, the TSS levels in chili peppers treated with NP and NC showed minimal increases, demonstrating the protective role of the chitosan matrix, enhanced by guava oil, in slowing down biochemical activities that would normally lead to a rise in TSS, such as the breakdown of complex carbohydrates into simpler sugars. Chitosan nanoprecipitation without oil (NNP and NNC) also resulted in lower TSS levels compared to untreated peppers but not as low as those treated with guava oil. Untreated peppers experienced the sharpest increase in TSS, indicating faster metabolic activity and potential spoilage.

As observed in this study, González-Saucedo et al. (2019) similarly justified that the chitosan matrix incorporated with *Byrsonima crassifolia* extract forms a semipermeable film on the surface of the chili peppers, which acts as a barrier that regulates gas exchange and reduces water loss. Alpos and Bayogan (2023), Faramitha et al. (2022), and Taheri et al. (2020) also alluded that this barrier effect helps slow down the peppers' respiration rate and metabolic activity, ultimately contributing to preserving their quality. Furthermore, Alpos and Bayogan (2023), Faramitha et al. (2022), and Taheri et al. (2020) posited in their works that the reduced respiration rates and metabolic activity can slow down the breakdown of complex carbohydrates into simpler sugars, a process that would normally lead to a rise in TSS. This is consistent with the observation that untreated peppers experience a sharper increase in TSS, indicating faster metabolic activity and spoilage.

The firmness of fruits and vegetables typically decreases during postharvest storage. This trend was clearly observed in chili peppers, but the use of chitosan nanoprecipitated with guava oil demonstrated the effectiveness of various treatments in maintaining fruit texture over time. Figure 10 highlights that untreated samples (CTRL) experienced a significant loss of firmness, dropping from 26.70 $\pm$ 0.63 N on Day 7 to 0.66 $\pm$ 0.12 N by Day 28. In contrast, samples treated with nanoprecipitation materials, such as NC and NP, maintained higher firmness, with NP-treated samples recording 53.90 $\pm$ 2.00 N on Day 7 and 30.95 $\pm$ 0.27 N on Day 28. These results suggest that treatments using nanomaterials significantly mitigate the loss of firmness in chili peppers.

Firmness is a critical attribute that impacts the visual attractiveness and shelf life of fruits. Similar studies show that the reduction in firmness, particularly in untreated fruits (CTRL), may be attributed to the enzymatic activity of pectinesterase, pectinmethylesterase, and polygalacturonase, leading to the breakdown of cell wall structures (Chen et al. 2015). This degradation accelerates in the presence of fungal organisms. However, the application of NP and NC has demonstrated the ability to slow down this softening process as seen in NP-treated fruits, which retained greater firmness. This effect is likely due to the antimicrobial properties of the treatments, a phenomenon that has also been observed with nanocoating on bell peppers (Correa-Pacheco et al. 2021).

The study investigates the effect of chitosan nanoprecipitation integrated with guava oil (via the solvent displacement method (NP) and nanoelaboration (NC)) and chitosan nanoprecipitate without oil (via the solvent displacement method (NNP) and nanoelaboration (NNC)) on the pH of chili pepper. The findings indicate that NP-coated and NC-coated fruits exhibited a comparatively slower reduction in pH, attributed to decreased cellular membrane leakage and the retention of organic acids, which serve as crucial modulators in various biochemical processes. This protective mechanism is consistent with similar studies by Arnon-Rips and Poverenov (2016); Das et al. (2021); R. Sharma et al. (2024); Yaman and Bayoindirli (2002), which established the effect of nanomaterials synthesized from edible coatings, such as chitosan, loaded with essential oils from plants, such as guava, in retaining organic acids and modulating biochemical processes. These coatings stabilize pH by acting as barriers to moisture loss and gas exchange, suppressing microbial proliferation, and conserving organic acid content. Additionally, the antimicrobial and antioxidant properties of guava essential oil help maintain physicochemical quality, delay senescence, and extend shelf life (Naseer et al. 2018).

This study also examines the impact of chitosan nanoprecipitation integrated with guava oil (via the solvent displacement method (NP) and nanoelaboration (NC)) and chitosan nanoprecipitate without oil (via the solvent displacement method (NNP) and nanoelaboration (NNC)) on the electrolyte leakage of chili pepper, which influences the ionic conductance. The efflux of ions, specifically K^+ and Na^+, serves as a marker of membrane degradation resulting from oxidative stress, leading to cellular damage through ion leakage from compromised plasma membranes (Huang et al. 2021; Khaliq et al. 2016). Chili peppers treated with NP and NC exhibited significantly lower ion leakage compared to those treated with CTRL, NNP, or NNC. The control group showed peak ion efflux after 28 days of storage, with NNC- and NNP-treated chili peppers displaying higher efflux values relative to NP and NC treatments. In contrast, NP and NC treatments resulted in reduced ion leakage from cellular structures. These results suggest that NP and NC treatments may enhance membrane integrity and extend the postharvest shelf life of chili pepper fruits by minimizing uncontrolled ion movement across cellular membranes (Saberi Riseh et al. 2023; Suryadi et al. 2017).

The ability of fruits, such as chili pepper, to maintain their antioxidant capacity has been well documented using several antimicrobial agents, including nanochitosan integrated with essential oils such as guava oil (A. Ali et al. 2015; González-Saucedo et al. 2019; Taheri et al. 2020). This is quantified through the assessment of the percentage inhibition of DPPH radicals. The works of A. Ali et al. (2015); Barik et al. (2024); Chaudhari et al. (2023); M. S. Nair et al. (2020); and A. Nair et al. (2022) further supported this assertion, stating that nanomaterials, such as nanochitosan, significantly reduce oxidative degradation by acting as oxygen barriers. Importantly, the incorporation of essential oils, such as guava oil, into these coatings enhances their efficacy, improving

antioxidant activity in fruits, such as oranges, by minimizing oxidative stress.

Chitosan has been identified as an effective abiotic elicitor that can induce disease resistance in fruits by promoting the production of reactive oxygen species (ROS), which in turn affects antioxidant levels. This process is crucial for maintaining the nutritional and functional properties of fruits, such as chili peppers (Alpos and Bayogan 2023). Studies by N. Kumar et al. (2021) and Manzoor et al. (2023) highlight the synergistic effects of nanomaterials synthesized from edible coatings, such as chitosan, in enhancing antioxidant capacity when incorporated into essential oils such as guava oil. The small size and low molecular weight of nanochitosan, when combined with guava oil, are expected to increase the surface area relative to their volume, making them biologically active and improving the bioavailability of active ingredients (Gomes et al. 2017). This controlled-release mechanism helps preserve the antioxidant capacity of the fruit, significantly contributing to antioxidant activity by neutralizing free radicals and preventing oxidative damage. This is essential for maintaining the fruit's quality (Pateiro et al. 2021). Additionally, a similar finding was reported by Mohammadi et al. (2016), who demonstrated that nanochitosan incorporated with *Zataria multiflora* helped preserve the antioxidant activity of cucumber during storage, delaying deterioration and nutrient loss.

This study investigated the impact of chitosan nanoprecipitation integrated with guava oil (via the solvent displacement method [NP] and nanoelaboration [NC]) and chitosan nanoprecipitation without oil (via the solvent displacement method [NNP] and nanoelaboration [NNC]) on the browning index of chili peppers, a key quality indicator of visual appeal and freshness. Chitosan nanoparticles mitigate enzymatic browning by forming a semipermeable barrier that limits oxygen diffusion, reducing the activity of browning-related enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) (Safitri et al. 2021; Sui et al. 2023). The incorporation of essential oils, such as guava oil, which is rich in bioactive compounds, such as flavonoids, terpenoids, and antioxidants, is expected to further suppress oxidative stress and enzymatic reactions (Pinho et al. 2014; Shaaban et al. 2012).

Nanochitosan has been shown to help retain moisture and reduce desiccation, a precursor to browning (Duan et al. 2019; J. Wang et al. 2024). Essential oils, such as guava oil, have hydrophobic properties that minimize water loss during storage, further contributing to freshness (Tongnuanchan and Benjakul 2014). The synergistic antioxidant activity of nanochitosan and guava oil neutralizes free radicals, limiting oxidative reactions and delaying senescence in postharvest produce (M. S. Nair et al. 2020; Yuan et al. 2016). This is further supported by studies emphasizing the role of nanochitosan and essential oils in reducing oxidative stress in fruits such as chili peppers (Hernández-López et al. 2020; Promwee and Matan 2025; Taheri et al. 2020).

The impact of chitosan nanoprecipitation (NP) integrated with guava oil and nanoelaboration (NC), as well as chitosan nanoprecipitation without oil (NNP and NNC), on the total phenolic content of chili peppers demonstrates promising advancements in postharvest preservation (Lattanzio and Botanik 2003). Phenolic compounds are essential secondary metabolites with antioxidant properties that influence the sensory and nutritional quality of fruits and vegetables, including texture, flavor, and taste. Previous studies have shown that coating treatments enhance phenolic content during storage. For instance, Edirisinghe et al. (2014) reported a 20% increase in phenolic content and improved disease resistance in bell peppers coated with 2% chitosan, whereas Poverenov et al. (2014) observed increases of 16.86% and 14.9% in red bell peppers coated with gelatine and chitosan, respectively, after 21 days of storage at 7°C. Similarly, Cuadra-Crespo and Del Amor (2010) found a 45% increase in phenolic content in urea-treated bell peppers under comparable conditions.

Incorporating essential oils into chitosan-based systems further enhances phenolic production. Essential oils, such as guava oil, act as signaling molecules that induce mild stress, stimulating phenolic compound synthesis as part of a defensive response (Gao et al. 2014). This mechanism was observed in red bell peppers treated with chitosan nanoparticles containing *Heracleum persicum* essential oil, which exhibited significant increases in phenolic content during storage compared with untreated controls (Taheri et al. 2020). Shankar et al. (2021) posited that the activation of phenylalanine ammonia lyase and the release of essential oil vapors from composite films, such as nanochitosan incorporated with guava oil, are suggested as potential mechanisms for these effects.

In the case of chili peppers, integrating guava oil into chitosan nanoprecipitation (NP and NC) is expected to significantly enhance phenolic content during storage. This is attributed to the combined effects of chitosan's preservative properties and the bioactivity of guava oil. This synergy includes phenolic induction through mild stress alongside the antimicrobial and antioxidant properties of guava oil (Bhowmik et al. 2022). By contrast, chitosan nanoprecipitation without oil (NNP and NNC) led to lesser enhancement of phenolic content in chili pepper due to the absence of the essential oil. Even lesser activity in phenolic content was observed in chili peppers that were left untreated.

Enzymatic browning, induced by polyphenol oxidase (PPO), is a significant postharvest issue that affects the quality and shelf life of fruits, including chilli peppers. PPO catalyzes the oxidation of phenolic compounds to quinones, leading to undesirable browning and deterioration of flavor and color (N. Sharma and Bhandari 2021). This study evaluated the effect of chitosan nanoprecipitation integrated with guava oil (NP) via the solvent displacement method and chitosan nanoprecipitation integrated with guava oil (NC) via nanoelaboration, as well as chitosan nanoprecipitation without oil (NNP and NNC), on PPO activity in stored pathogen-induced chili peppers.

Pathogenic fungal, such as *R. stolonifer* and *C. gloeosporioides,* are known to exacerbate enzymatic browning in chili peppers by inducing oxidative stress and increasing PPO activity (Feng et al. 2022; X. Zhang et al. 2020). These fungi can trigger ROS accumulation and promote tissue damage, creating a conducive environment for PPO activation and phenolic oxidation. This highlights the influence of fungal infections on enzymatic browning during postharvest storage (H. Wang et al. 2018).

The results showed that chitosan nanoprecipitation significantly reduced PPO activity compared to the control. PPO activity increased in the control group over time, consistent with previous reports of oxidative stress-driven PPO escalation (Taheri et al. 2020) and the contributions of fungal-induced oxidative stress. However, in the NP and NC treatments, PPO activity was significantly lower, particularly in later storage stages, even in the presence of fungal pathogens.

The reduction in PPO activity can be attributed to the antioxidative properties of chitosan and its nanoformulation. Chitosan is known to inhibit enzymatic browning by chelating copper ions at the active sites of PPO (N. Sharma and Bhandari 2021). Additionally, the incorporation of guava oil enhanced this effect, likely due to its antioxidant properties, which reduce oxidative stress and the formation of ROS that activate PPO (Aly et al. 2022). These antioxidative effects potentially mitigated the prooxidant influence of *R. stolonifer* and *C. gloeosporioides*.

The NC and NP treatments likely improved the stability and controlled release of active ingredients, providing sustained effects throughout storage. In contrast, the NNP and NNC treatments, lacking guava oil, were less effective at reducing PPO activity, although still more effective than the control. These findings align with previous research indicating that chitosan nanoparticles incorporated with essential oils, such as guava oil, can modulate PPO activity by chelation and by acting as a barrier to oxygen (Hassan et al. 2021; Safitri et al. 2021; Taheri et al. 2020). Additionally, the antifungal properties of nanochitosan integrated with guava oil likely suppressed the growth of *R. stolonifer* and *C. gloeosporioides*, thereby indirectly reducing their contribution to PPO activation and browning.

Furthermore, this study investigates the impact of chitosan nanoprecipitation integrated with guava oil via solvent dispersion method (NP) and chitosan nanoprecipitation integrated with guava via the nanoelaboration method (NC) and without guava oil (NNP via the solvent dispersion method and NNC via the nanoelaboration method) on β-1,3-glucanase activity in chili peppers. Chitosan treatments are well-documented for enhancing plant defense mechanisms by inducing enzymes, such as β-1,3-glucanase. The enzyme fortify plant resistance against fungal infections, delay fruit ripening, and improve postharvest quality (Abdel-Rahman et al. 2021; Rajestary et al. 2021).

Several studies have established that chitosan enhances pathogen resistance by stimulating defense enzymes, reducing reactive oxygen species, and degrading fungal cell walls via β-1,3-glucanase (Walled Fouad 2023). In this study, NP and NC treatments boosted β-1,3-glucanase activity, whereas guava oil in NP and NC formulations amplified antimicrobial and antioxidative effects, thereby further increasing β-1,3-glucanase activity, likely through the activation of pathogenesis-related proteins and the strengthening of cell walls (Rosli et al. 2021; Ye et al. 2023). The study also showed smaller increases in β-1,3-glucanase activity in NNP and NNC treatments, with the lowest activity observed in the control treatment. These results are consistent with previous research, such as the work of Soleimani, Mostowfizadeh-Ghalamfarsa, and Zarei (2024), who found that encapsulating celery seed essential oil in chitosan nanoparticles (CSEO-LCNPs) enhanced β-1,3-glucanase gene expression in cucumber plants. Similar findings have been reported in strawberries (Dong and Wang 2017) and apples (W. Zhang et al. 2023), where chitosan incorporated with essential oils increased β-1,3-glucanase activity and helped delay fruit decay, as demonstrated by W. Zhang et al. (2023) using a Chitosan–Cinnamon essential oil composite coating on apple fruits. Furthermore, the increased β-1,3-glucanase activity observed in chili peppers aligns with the role of chitosan nanoencapsulated with essential oil, such as guava oil, in reducing enzymatic degradation during storage, thus enhancing resistance to fungal invasion as noted by (Dhanasekaran et al. 2024; W. Zhang et al. 2022).

The study investigated the effects of chitosan nanoprecipitation integrated with guava oil via solvent dispersion (NP) and nanoelaboration (NC) methods, as well as chitosan nanoprecipitation without guava oil (NNP and NNC), on enhancing phenylalanine ammonia-lyase (PAL) activity in chili pepper plants inoculated with *Rhizopus stolonifer* and *Colletotrichum gloeosporioides*. PAL, a key enzyme in the phenylpropanoid pathway, plays a crucial role in synthesizing phenolics and other defense-related compounds essential for plant resistance against pathogens (Yadav et al. 2020).

In control (CTRL) and nanochitosan-only (NNP and NNC) treatments, PAL activity exhibited a characteristic pattern of an initial increase followed by a decline, consistent with previous findings on pathogen-induced stress. In contrast, treatments incorporating guava oil (NP and NC) maintained elevated PAL activity over time. This suggests that guava oil enhances the plant's defense mechanisms more effectively than control (CTRL) and nanochitosan-only (NNP and NNC) treatments, potentially due to its antimicrobial properties working synergistically with chitosan to upregulate PAL and other defense responses (Bhatt et al. 2024; Landi et al. 2021).

The presence of guava oil not only sustained PAL activity but also modulated other defense-related enzymes, improving the plant's resistance to *C. gloeosporioides* and *R. stolonifer*. This enhanced activity led to the production of protective compounds, such as lignin, which reinforces the plant cell wall and impedes pathogen invasion. Additionally, the consistent upregulation of PAL suggests the activation of other defense pathways, including the production of phytoalexins and secondary metabolites critical for plant immunity (Govender et al. 2017; Miedes et al. 2014; Ninkuu et al. 2023).

Interestingly, the NP and NC treatments exhibited a distinct PAL activity profile compared to chitosan-only treatments, indicating that guava oil alters the plant's response by modulating pathogen recognition and defense gene expression. Studies highlight the role of bioactive compounds in influencing the phenylpropanoid pathway and related defense genes, further supporting the observed effects (Landi et al. 2021; Miedes et al. 2014; Ninkuu et al. 2023).

Moreover, the formulation strategy appeared to influence the treatments' efficacy. The solvent dispersion method (NP) may have enabled better integration of guava oil into the chitosan

matrix, resulting in improved stability and a controlled release of bioactive compounds. On the other hand, the nanoelaboration method (NC) likely produced different particle characteristics, affecting release rates and interactions with plant defense systems. These differences underscore the importance of optimizing formulation strategies for designing effective chitosan-based treatments to enhance plant resistance (Karamchandani et al. 2024; Riseh et al. 2022; Yu et al. 2021). In addition, this study demonstrates that the efficacy of the treatments contributes to the reduced deterioration of the fruits at room temperature, due to the ability of NP and NC to protect chili peppers without refrigeration. This is particularly significant when compared to the well-documented benefits of cold chain storage, which may be challenging to implement in tropical regions owing to the limited financial capacity of stakeholders (Kumari et al. 2024).

This preliminary investigation evaluated the toxicity of chitosan nanoparticles incorporating guava oil, synthesized via solvent displacement (NP) and nanoelaboration (NC) methods, using the brine shrimp lethality assay. The results demonstrated that both NP and NC formulations exhibited higher toxicity toward brine shrimp compared to chitosan nanocapsules without oil, prepared using solvent displacement (NNP) and nanoelaboration (NNC) techniques, as well as the control group. This suggests that the phytoconstituents in guava oil contribute to the increased lethality observed in NP and NC formulations.

However, according to Meyer et al. (1982), the observed lethality falls within the recommended range of ≥ 1000 μg/mL, indicating that natural antimicrobials within this range are considered nontoxic. This study corroborates earlier works by Fasola et al. (2011) and Weli et al. (2019) that equally confirmed the nontoxicity of guava essential oil and guava leaf extract, respectively.

5 | Conclusion

Chitosan-based nanopolymer coatings enriched with guava oil present a promising approach for postharvest preservation by harnessing the biopolymeric properties of chitosan and the bioactive efficacy of essential oils. These innovative coatings effectively mitigate fruit rot caused by fungal pathogens, such as *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, whereas simultaneously enhancing the biochemical defenses of chili peppers (*Capsicum frutescens*). The process of nanoencapsulating guava oil through chitosan nanoprecipitation yields a biodegradable and biocompatible coating that facilitates the controlled release of antifungal agents. This mechanism results in a significant reduction in disease incidence and severity, an extension of shelf life characterized by retained firmness and stable pH over a 28-day storage period, and an increase in antioxidant capacity, along with heightened activities of defense-related enzymes. Furthermore, the nontoxic nature of this coating positions it as a natural and eco-friendly alternative to synthetic preservatives, aligning with the growing demand for sustainable postharvest technologies.

Author Contributions

Oluwagbenga Adeogun: writing – original draft, writing – review and editing, resources, methodology, investigation, data curation, conceptualization, software, supervision **Mujeeb Lawal:** writing – review and editing, investigation, data curation. **Olufunke Nejo:** resources, investigation, data curation, conceptualization. **Adedotun Adekunle:** writing – review and editing, resources, investigation, formal analysis, conceptulaization.

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Ethics Statement

We, the authors confirm that this research was conducted in accordance with all applicable ethical standards that all authors contributed to this work, and that appropriate protocols for data collection, analysis, and reporting were strictly followed.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available upon request.

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