

Effect of Eugenol Nanoemulsion on Microbial Load and Sensory Properties of Fresh-Cut Papaya During Storage

Efecto de la Nanoemulsión con Eugenol sobre la Carga Microbiana y Propiedades Sensoriales de Papaya Fresca Cortada Durante el Almacenamiento

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ABSTRACT

Fresh-cut fruits are valued for their convenience and nutritional benefits; however, their shelf life is limited by physicochemical and microbiological deterioration after peeling and cutting. Among emerging preservation strategies, essential oils are recognized for their antimicrobial activity; however, their direct use is hindered by their high volatility, low water solubility, and strong sensory impact. Nanoemulsions offer a promising alternative to overcome these limitations. In this study, eugenol nanoemulsions were prepared via sonication and evaluated for their effects on microbial load and sensory attributes of fresh-cut papaya stored at 4 °C for 15 d. The nanoemulsions exhibited a hydrodynamic radius of 9.78-210 nm and zeta potentials between -5.29 and -7.44 mV. Physical stability (creaming index) ranged from 0 % (0.4 % eugenol) to 100 % (0.6 % eugenol) at the end of the storage. *In vitro* assays showed that eugenol nanoemulsions reduced *Listeria monocytogenes* and *Salmonella Typhimurium* by 1.2-5.4 Log CFU/mL. When applied to papaya inoculated with each pathogen, eugenol nanoemulsions reduced counts by 1.5-5 Log CFU/mL, depending on dose, and maintained this effect for 15 d. However, the nanoemulsions affected the aroma and taste of papayas. In conclusion, eugenol nanoemulsions effectively reduced pathogenic contamination, demonstrating their potential as a natural preservation strategy, provided that sensory modifications are optimized for consumer acceptance.

Keywords: Minimally processed fruits; *Listeria monocytogenes*; *Salmonella Typhimurium*; essential oils; encapsulation.

RESUMEN

Las frutas recién cortadas son valoradas por su conveniencia y valor nutricional; sin embargo, su vida útil está limitada por la degradación fisicoquímica y microbiológica. Entre las estrategias de conservación, los aceites esenciales destacan por su actividad antimicrobiana, aunque su aplicación se ve restringida por su alta volatilidad, baja solubilidad y fuerte

impacto sensorial. Las nanoemulsiones representan una alternativa para superar estas limitaciones. En este estudio, se formularon nanoemulsiones de eugenol por sonicación y se evaluó su efecto sobre la carga microbiana y los atributos sensoriales de papaya cortada almacenada a 4 °C durante 15 d. Las nanoemulsiones presentaron tamaños de partícula de 9.78-210 nm y potencial zeta entre -5.29 y -7.44 mV. La estabilidad física (índice de cremosidad) varió de 0 % a 100 % (0.6 % eugenol) dependiendo de la dosis de eugenol. Las nanoemulsiones de eugenol redujeron *Listeria monocytogenes* y *Salmonella Typhimurium in vitro* y en papayas inoculadas con cada patógeno, manteniendo este efecto durante 15 días. Sin embargo, las nanoemulsiones alteraron el aroma y el sabor de las papayas. En conclusión, las nanoemulsiones de eugenol redujeron la contaminación patógena, demostrando su potencial como estrategia de conservación natural, siempre que las modificaciones sensoriales se optimicen para la aceptación del consumidor.

Palabras clave: Frutas mínimamente procesadas; *Listeria monocytogenes*; *Salmonella Typhimurium*; aceites esenciales; encapsulación.

INTRODUCTION

The growing consumer preference for healthier, natural, and functional foods has significantly increased the consumption of fresh fruits, especially ready-to-eat products. In this context, fresh-cut fruits (FCFs) are experiencing increasing demand due to their convenience, freshness, and nutritional benefits, making them one of the fastest-growing segments of the food market. FCFs are defined as fruits that have undergone minimal physical modification, such as washing, peeling, or slicing, without thermal or chemical treatments that would significantly alter their nutritional or sensory properties (Iturralde-García *et al.*, 2022). These products provide essential nutrients, including fiber, vitamins, minerals, and antioxidants, which are linked to the prevention of chronic

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non-communicable diseases such as obesity, type 2 diabetes, and cardiovascular disorders (Jideani *et al.*, 2021).

Papaya (*Carica papaya* L.), a widely consumed tropical fruit, is rich in vitamin C, provitamin A, digestive enzymes, and bioactive compounds (Dotto and Abihudi, 2021). However, when processed as FCF, papaya becomes highly perishable due to mechanical damage that occurs during peeling, cutting, and handling, which accelerates respiration, enzymatic browning, and microbial growth, leading to rapid deterioration and a short shelf life (Iturralde-García *et al.*, 2022). These physicochemical and microbiological changes not only reduce sensory quality but also pose potential safety concerns due to the proliferation of spoilage and pathogenic microorganisms. According to reports from the United States Centers for Disease Control and Prevention (CDC), *Listeria monocytogenes* and *Salmonella* Typhimurium are the main pathogens responsible for foodborne outbreaks in 2025 linked to FCFs and ready-to-eat foods, underscoring the urgent need for effective natural preservation strategies to control these microorganisms. *L. monocytogenes* can grow at refrigeration temperatures and is particularly dangerous for vulnerable populations, while *S. Typhimurium* is a common cause of gastroenteritis worldwide. These pathogens can contaminate fruits during harvesting, processing, or handling, posing a significant risk in ready-to-eat products (Luciano *et al.*, 2023).

Traditional chlorine-based sanitizers are used before the cut operation and may reduce microbial load, but they raise concerns about toxicity and resistance (Zaman *et al.*, 2024). Because FCFs cannot be heat-treated, strategies are being sought to extend their shelf life beyond refrigeration, which can cause chilling damage in tropical fruits (Giannakourou and Tsironi, 2021; Wongs-Aree *et al.*, 2024). For these reasons, effective preservation strategies that ensure microbial safety without compromising sensory quality, thereby extending the shelf life of FCFs, have become a significant challenge in food technology.

Among emerging preservation approaches, essential oils have garnered considerable attention due to their broad-spectrum antimicrobial properties and Generally Recognized as Safe (GRAS) status, making them attractive as natural alternatives to synthetic preservatives (Yousuf *et al.*, 2021). Eugenol, a phenylpropanoid contained in clove, cinnamon, and basil oils, exerts antimicrobial activity against various foodborne pathogens by altering cell membrane permeability and disrupting microorganism integrity (Silva *et al.*, 2024). However, its direct use in foods is limited due to its high volatility, low water solubility, and intense aroma, which can negatively affect sensory acceptance and stability during storage.

To address these issues, nanoemulsions have been proposed as an effective delivery system for essential oils. Nanoemulsions, colloidal systems with droplet sizes ranging from 20 to 200 nm, have emerged as a promising technology in the food industry. Due to their small droplet size and large surface area, nanoemulsions enhance solubility, improve

physicochemical stability, and minimize their sensory impact (Pandey *et al.*, 2024). These structures enable controlled release and enhanced interaction with the microbial surface, thereby improving the antimicrobial activity of bioactive compounds such as eugenol (Pavoni *et al.*, 2020). Furthermore, their transparent appearance and kinetic stability make them suitable for incorporation into aqueous food systems, such as fresh-cut fruits. Recent studies have reported that eugenol nanoemulsions can effectively inhibit microbial growth and extend shelf life in products such as strawberries, apples, and mangoes, without compromising quality (Prakash *et al.*, 2018; da Silva *et al.*, 2020). Considering these advantages, this study aimed to develop and characterize an eugenol-based nanoemulsion and to evaluate its effects on microbial load and sensory attributes of fresh-cut papaya during refrigerated storage. The findings are expected to contribute to the development of natural, efficient, and consumer-acceptable preservation systems for fresh-cut tropical fruits.

MATERIALS AND METHODS

Preparation for eugenol nanoemulsion

Nanoemulsions of eugenol were formulated via ultrasound-assisted emulsification. Initially, a 2.0 % (w/w) solution of the non-ionic surfactant Tween 80 (T80) was prepared by gradually adding it to deionized water at room temperature, followed by gentle manual stirring for 1 minute to ensure complete dissolution. This aqueous phase served as the base for all formulations, which were subsequently prepared by directly mixing the aqueous phase with the oily phase containing eugenol (Sigma-Aldrich, 99 % purity) at concentrations of 0, 0.4, 0.5, 0.55, and 0.6 % (w/w), without prior mechanical stirring. This biphasic mixture was immediately subjected to direct sonication for 5 minutes at 40 % amplitude using ultrasonic probe equipment at 500 W (VCX-500, Sonics & Materials, E.U.). The temperature was controlled throughout the process to prevent losses due to the volatilization of the active compound. The resulting nanoemulsions were stored in amber glass vials at room temperature and protected from light until their physicochemical characterization and application in food matrices.

Characterization of nanoemulsions

To evaluate the colloidal stability and structural properties of eugenol-based nanoemulsions, various analytical methods were employed. Particle size was determined using the Dynamic Light Scattering (DLS) technique with a Zetasizer ALV 5000 (Malvern Instruments) instrument, configured at a 90° detection angle. This instrument determined the hydrodynamic radius of the droplets in the emulsion, expressed in nanometers (nm). Before each measurement, the samples were diluted in deionized water and placed in clean, bubble-free optical analysis cells to avoid interference. Measurements were performed at room temperature, and at least three independent readings were obtained per sample.

The zeta potential (ζ) was determined to evaluate the colloidal stability of the formulated nanoemulsions. The

samples were pre-diluted in deionized distilled water (1:20 v/v) to avoid multiple dispersions and minimize interference. They were then introduced into a transparent capillary-type sample cell designed for this type of electrokinetic measurement. The analysis was performed at 25 °C, under controlled pH and conductivity conditions. Each sample was measured in triplicate, and the average readings were reported in millivolts (mV). Absolute ζ -values greater than ± 30 mV were considered indicative of good colloidal stability, while values close to 0 mV suggest a greater tendency to aggregation (Araujo *et al.*, 2021).

Creaming index

The creaming index is a physical indicator that allows the evaluation of an emulsion's kinetic stability over time. The creaming index was calculated by direct observation of the system over 15 days at room temperature. Glass test tubes were used to store 10 mL of each formulation. On days 0, 5, 10, and 15, the height of the separated phase (cream) was measured relative to the total height of the system using the following formula:

$$IC = \left(\frac{H_c}{H_t} \right) \times 100$$

where H_c corresponds to the height of the separated upper layer and H_t to the total height of the emulsified system (Osanloo *et al.*, 2017).

Antibacterial activity of eugenol-based nanoemulsions

The antibacterial activity of the eugenol nanoemulsions was evaluated by viable cell counting. Strains of *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 and *Listeria monocytogenes* ATCC 7644 were reactivated in Mueller-Hinton broth and incubated at 37°C for 19 h. The bacterial suspensions were adjusted to an optical density corresponding to approximately 1×10^8 colony-forming units per milliliter (CFU/mL), as measured at 600 nm using a spectrophotometer. For each treatment, 900 μ L of nanoemulsion and 100 μ L of bacterial suspension were combined in sterile tubes to achieve a final concentration of 1×10^6 CFU/mL. The treatments evaluated were the control (bacteria without eugenol-based nanoemulsion), the blank (Tween 80 + water), and nanoemulsions with different concentrations of eugenol (0.4 %, 0.5 %, 0.55 %, and 0.6 %). Samples were incubated at 37 °C for 24 h, then diluted in sterile saline and plated to determine viable cell counts. All assays were performed in triplicate, and results were expressed as the mean of Log CFU/mL $\pm$ standard deviation.

Preparation of fresh-cut papaya and application of eugenol-based nanoemulsions

Commercially ripe papaya (*Carica papaya* L.) fruits were selected from a local supermarket in Hermosillo, Sonora, Mexico. The fruits were washed with potable water and disinfected by immersion in 150 min sodium hypochlorite for 5 min. They were then rinsed twice with sterile distilled water

and allowed to dry for 15 min in a clean, previously sanitized area. The papayas were peeled and cut into cubes of approximately 1 cm³, ensuring uniformity in shape and weight. They were placed in a clean tray under aseptic conditions for further analysis of bacterial load.

Inoculation and analysis of bacterial load in papaya treated with eugenol-based nanoemulsions

The fruit pieces were placed in sterile beakers and inoculated separately with *L. monocytogenes* ATCC 7644 or *S. Typhimurium* ATCC 14028 by immersing the papaya cubes in 5 mL of bacterial suspension containing approximately 1×10^5 CFU/mL for 5 min. After inoculation, the samples were air-dried in a biosafety cabinet for 15 minutes under sterile conditions to allow bacterial adherence. Subsequently, eugenol nanoemulsions at different concentrations (0.4-0.6 % w/w) were applied to fresh-cut, inoculated papayas by immersion for 5 min, followed by a 30 min drying period. The aqueous phase (Tween 80 + deionized water) served as the blank, and deionized water without nanoemulsion was used as the control. The different treated papayas (10 g) were transferred to sterile plastic containers and stored at 4°C for 15 days. At predetermined intervals (0, 5, 10, and 15 days), 10 g of each sample was aseptically transferred to sterile bags containing 90 mL of saline solution (0.85 % NaCl), homogenized for 2 min, and then serially diluted. Appropriate dilutions were plated on LB agar using the spread plate method to enumerate viable *L. monocytogenes* and *S. Typhimurium* cells, and the plates were then incubated at 37°C for 24 h. All experiments were performed in triplicate, and the results were expressed as the mean of Log CFU/g $\pm$ standard deviation.

Sensory analysis of fresh-cut papaya treated with eugenol nanoemulsions

A sensory evaluation was conducted to determine the impact of eugenol nanoemulsions on the organoleptic attributes of fresh-cut papaya using a consumer-level test. Sensory evaluation was performed on the first day of storage at 4°C. Four sample groups were prepared: a control (without nanoemulsions), a blank (Tween 80 + water), and two nanoemulsion groups containing eugenol at 0.4 % and 0.6 %. The evaluation involved 36 untrained panelists (men and women aged 18 - 30) who provided informed consent and were unaware of the treatments. All participants received a brief orientation on the evaluation procedure and the use of the sensory scale. A 9-point hedonic scale was used, with 9 the highest and 1 the lowest, labeled "extremely liked" and "extremely disliked," respectively. The attributes analyzed were aroma, taste, texture, color, and overall acceptability of the treated cut papayas.

Statistical analysis

This study used a completely randomized experimental design to analyze the effect of different eugenol concentrations in nanoemulsion formulations on the hydrodynamic radius (nm), ζ -potential (mV), bacterial load (Log CFU/mL), and

level of taste, texture, color, aroma, and overall acceptance of fresh-cut papaya. One-way analysis of variance (ANOVA) was performed to determine significant differences between treatments ($p \leq 0.05$). When differences were found, the Tukey-Kramer test ($p \leq 0.05$) was used to identify statistically distinct groups. Statistical analysis was performed using NCSS statistical software version 2011.

RESULTS AND DISCUSSION

Synthesis and characterization of eugenol nanoemulsions

The nanoemulsions were characterized based on three key parameters: hydrodynamic radius, ζ -potential, and creaming index. These indicators are essential for assessing the stability and functional performance of emulsion systems. Ultrasonic cavitation enhanced oil droplet fragmentation, allowing the formation of spherical nanostructures with diameters below 291 nm and good visual stability. Results showed that the hydrodynamic radius ranged from 9 to 210 nm, depending on eugenol concentration. This trend may be attributed to the gradual saturation of the surfactant, which, as oil-phase loading increases, may reduce its capacity to stabilize all formed droplets, leading to the formation of larger structures.

As shown in Table 1, particle size decreased upon the incorporation of eugenol. The control formulation (without eugenol) exhibited a mean hydrodynamic radius of 291.86 nm. With 0.4 % eugenol, the radius decreased significantly to 9.78 nm, suggesting enhanced emulsification efficiency, likely due to molecular interactions between eugenol and Tween 80 that promoted a more homogeneous and stable droplet distribution. Interestingly, intermediate concentrations (0.5 % and 0.55 %) resulted in a moderate increase in the hydrodynamic radius, followed by a reduction to 209.78 nm at 0.6 % eugenol. This behavior suggests interfacial saturation effects that may play a role in droplet stabilization at higher oil concentrations. At low oil levels, the presence of eugenol likely improves emulsification by interacting with Tween 80 and facilitating droplet disruption, which explains the sharp reduction to ~ 10 nm at 0.4 %. However, as the oil fraction increases, the amount of surfactant becomes relatively insufficient to cover the newly created interfacial area,

leading to partial coalescence and an increase in droplet size. This effect has been described for nanoemulsions containing citrus oils, thyme oil, and clove-derived components, where an optimal surfactant-to-oil ratio produces the smallest droplets, but further increases in oil result in larger particle sizes due to interfacial saturation and steric limitation of the surfactant layer (Salvia-Trujillo *et al.*, 2013; Assadpour and Jafari, 2019).

All values were within the functional threshold for nanoemulsions, typically defined as systems with particle sizes below 500 nm (Sharma *et al.*, 2022). Maintaining droplets within this range is crucial, as smaller droplets provide a higher specific surface area, promoting more efficient diffusion of the bioactive compound (eugenol) and greater interaction with microbial cell membranes (Fu *et al.*, 2023). Reduced droplet size is desirable not only for improved physical stability (less coalescence or sedimentation), but also for enhancing the functional performance of essential oil nanoemulsions, particularly their antimicrobial efficacy (Fu *et al.*, 2023). Comparable droplet sizes have been reported for carvacrol and rosemary essential oil nanoemulsions stabilized with Tween 80 and produced by sonication, with mean diameters of 102.11 nm and 132.58 nm, respectively (Elsherif *et al.*, 2024). In contrast, our results are lower compared to those reported by Luciano *et al.* (2023), who reported a droplet size of 194 nm for citral nanoemulsions prepared using Tween 80 and a catastrophic phase inversion method. Similarly, our results are comparable to those of the carvacrol nanoemulsions and rosemary essential oil nanoemulsions prepared using Tween 80 and sonication, which showed average droplet sizes of 102.11 nm and 132.58 nm, respectively.

The ζ -potential values of eugenol nanoemulsions are presented in Table 1. All formulations exhibited moderately negative surface charges, ranging from -5.29 to -9.87 mV, with statistically significant differences among treatments ($p \leq 0.05$). The sample composed of Tween 80 in water without eugenol showed the most negative ζ -potential (-9.87 ± 0.91 mV). This value reflects the adsorption of anionic species or ionized groups at the droplet interface, even in the presence of a non-ionic surfactant. The relatively high negative charge suggests sufficient electrostatic repulsion to confer some degree of physical stability; however, this formulation also presents a broad particle size distribution, indicating limited stabilization efficiency in the absence of an oil phase. The introduction of eugenol at 0.4 % led to a significant decrease in the absolute value of the ζ -potential (-5.29 ± 0.81 mV), likely due to the partial neutralization or masking of interfacial charges by eugenol molecules, which may interact with surfactant head groups or alter the interfacial composition. This reduction in surface charge could influence droplet interactions and stability, though in this case, the small droplet size likely compensated for the reduced electrostatic repulsion.

At higher concentrations (0.5 % to 0.6 % eugenol), the ζ -potential slightly increased in absolute value, ranging from -6.69 to -7.44 mV. These eugenol nanoemulsions exhibited a negatively charged interface, which can be attributed to the

Table 1. Hydrodynamic radius and zeta potential of eugenol nanoemulsions.
Tabla 1. Radio hidrodinámico y potencial zeta de las nanoemulsiones con eugenol.

Treatment	Zeta ζ -Potential (mV)	Hydrodynamic Radius (nm)
Blank (Tween 80 + water)	-9.87 ± 0.91^a	291 ± 417.93^a
Nanoemulsion 0.4 % eugenol	-5.29 ± 0.81^b	9.78 ± 0.18^b
Nanoemulsion 0.5 % eugenol	-7.44 ± 0.78^c	103.06 ± 2.30^c
Nanoemulsion 0.55 % eugenol	-7.38 ± 0.76^c	136.86 ± 3.76^c
Nanoemulsion 0.6 % eugenol	-6.69 ± 0.93^c	209.78 ± 1.27^c

Values are expressed as mean $\pm$ standard deviation. Different superscript letters indicate significant differences ($p \leq 0.05$).

fact that Tween 80 is a non-ionic emulsifier that typically displays negative ζ -potential values in nanoemulsions (Salvia-Trujillo *et al.*, 2013). Although the ζ -potential values obtained (-5.29 to -9.87 mV) are below the conventional ± 30 mV stability threshold, this behavior is typical of nanoemulsions stabilized with Tween 80. In such systems, stability is mainly governed by steric repulsion provided by the long polyoxyethylene chains of Tween 80 rather than by electrostatic interactions (Prakash *et al.*, 2020; Fuentes *et al.*, 2021). Similar observations have been reported by Luciano *et al.* (2023), who described how Tween 80 in citral-based nanoemulsions exhibits low ζ -potential values (ranging from -6.09 to -11.90 mV) yet remains physically stable due to steric stabilization mechanisms.

The values observed across these formulations suggest that eugenol concentration influences surface charge up to a certain point, beyond which the effect stabilizes, possibly due to saturation at the oil–water interface. Overall, the moderately negative ζ -potential values indicate a balance between electrostatic and steric stabilization, which contributed to the physical stability observed across the formulations. The findings highlight the importance of optimizing both the bioactive compound concentration and the surfactant system to achieve colloidal stability without compromising functional performance. Dutra *et al.* (2022) reported that the concentrations of essential oil and Tween 80 are key factors influencing droplet size. Surfactant-to-oil ratios up to 3:1 can yield droplets smaller than 180 nm, exhibiting enhanced antibacterial activity compared to pure oils. These improved properties are attributed to the droplet's size and composition, as the balance between the hydrophobic core and hydrophilic shell directly affects the antimicrobial mechanism.

Stability of eugenol nanoemulsion

The creaming index (%) of the nanoemulsions with varying eugenol concentrations was monitored over 15 days at room temperature to study their stability. The results are shown in Table 2. Creaming is a physical destabilization phenomenon where oil droplets migrate upward due to density differences between the dispersed and continuous phases, and it is commonly used as an indicator of nanoemulsion stability. On day 0, all formulations, including the control and all eugenol-containing nanoemulsions, exhibited no visible phase separation (creaming index = 0 %), indicating that the emulsification process was initially successful in producing homogeneous and stable systems. However, differences in creaming behavior emerged over time. The control sample (Tween 80 without eugenol) and the formulation with 0.4 % eugenol remained visually stable throughout the 15-day storage period, showing no signs of creaming. This suggests that a low eugenol concentration, combined with adequate surfactant coverage, yielded kinetically stable nanoemulsions.

In contrast, formulations containing 0.5 %, 0.55 %, and 0.6 % eugenol exhibited progressive destabilization. By day 5, noticeable creaming was observed in these samples, with

Table 2. Creaming index (%) of nanoemulsions with different concentrations of eugenol during 15 days of storage at 4 °C.

Tabla 2. Índice de cremado (%) de nanoemulsiones con diferentes concentraciones de eugenol durante 15 días de almacenamiento a 4 °C.

Treatment	Creaming index (%)			
	Storage days			
	0	5	10	15
Blank (Tween 80 + water)	0	0	0	0
Nanoemulsion 0.4 % eugenol	0	0	0	0
Nanoemulsion 0.5 % eugenol	0	15	50	85
Nanoemulsion 0.55 % eugenol	0	40	100	100
Nanoemulsion 0.6 % eugenol	0	50	100	100

the degree of creaming increasing with the eugenol concentration. The 0.5 % formulation reached an 85 % creaming index by day 15, whereas both the 0.55 % and 0.6 % formulations achieved complete phase separation (100 %) by day 10. This trend may be explained by the saturation of surfactant capacity at higher oil phase loads, resulting in insufficient stabilization of the increased number or size of droplets, which promotes droplet migration and aggregation over time. These results can be attributed to the ζ -potential; the 0.4 % eugenol nanoemulsion exhibited the lowest ζ -potential (-5.29 mV) and remained fully stable (creaming index = 0 %), indicating that steric stabilization by Tween 80 effectively compensated for the limited electrostatic repulsion. In contrast, higher eugenol concentrations (0.55 %) exhibited slightly more negative ζ -potentials (≈ -7 mV) but increased creaming, resulting in complete phase separation by day 10. This suggests that ζ -potential alone does not determine stability in non-ionic systems; at higher oil loads, surfactant saturation reduces interfacial coverage, leading to droplet coalescence. Overall, steric stabilization predominates at low eugenol levels, while insufficient surfactant-to-oil ratios at higher concentrations weaken both steric and electrostatic stabilization, promoting phase separation.

These findings highlight an apparent concentration-dependent destabilization effect. While moderate eugenol concentrations (e.g., 0.4 %) can maintain stability under cold storage conditions, higher concentrations appear to exceed the emulsifier system's stabilizing capacity, resulting in significant creaming. This emphasizes the importance of optimizing both surfactant-to-oil ratios and droplet size in nanoemulsion formulations to prevent physical instability during shelf life.

Antibacterial Activity of Eugenol-Containing Nanoemulsions

The antibacterial activity of eugenol-containing nanoemulsions was evaluated against two critical foodborne pathogens, *S. Typhimurium* and *L. monocytogenes*. Figure 1 shows the quantification of viable cells (Log CFU/mL) after treatment with the blank (Tween 80 + water) and nanoemulsions with different concentrations of eugenol (0.4 %, 0.5 %, 0.55 %, and 0.6 %), compared to a control (bacteria without treatment).

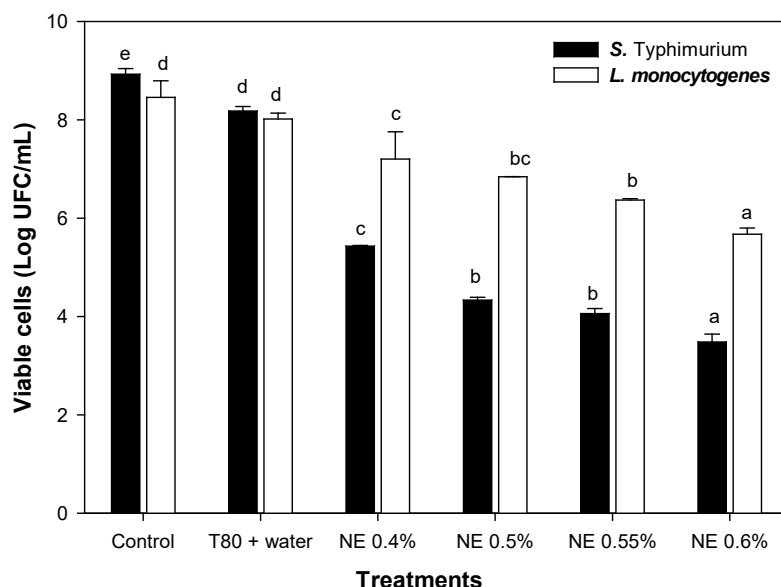


Figure 1. Effect of nanoemulsions (NE) with different eugenol concentrations on the viability of *Salmonella Typhimurium* and *Listeria monocytogenes*. The bars represent the mean $\pm$ standard deviation. Different letters indicate significant differences ($p \leq 0.05$).

Figura 1. Efecto de las nanoemulsiones (NE) con diferentes concentraciones de eugenol sobre la viabilidad de *Salmonella Typhimurium* y *Listeria monocytogenes*. Las barras representan los valores medios de $\pm$ desviación estándar. Letras diferentes indican diferencias significativas ($p \leq 0.05$).

The results showed that eugenol-containing nanoemulsions exhibit concentration-dependent inhibitory activity. In the control, both pathogens showed elevated counts, above 8.5 Log CFU/mL. The blank treatment (Tween 80 + water) did not produce a significant reduction ($p > 0.05$) in microbial populations compared to the control, confirming that the observed antimicrobial activity is specifically attributed to the presence of encapsulated eugenol.

The addition of 0.4 % eugenol nanoemulsion significantly reduced the populations of *S. Typhimurium* (6.0 Log CFU/mL) and *L. monocytogenes* (7.0 Log CFU/mL), with differences compared to the control ($p \leq 0.05$). As the eugenol concentration increased, progressive and significant reductions in the viability of both pathogens were observed. In particular, the formulation with 0.6 % eugenol achieved the greatest reductions, with cell viability values of 3.8 Log CFU/mL for *S. Typhimurium* and 5.1 Log CFU/mL for *L. monocytogenes*, showing differences compared to the other treatments ($p \leq 0.05$).

S. Typhimurium was observed to be more susceptible to the action of nanoencapsulated eugenol than *L. monocytogenes*. Although Gram-negative bacteria commonly exhibit greater resistance to antimicrobial compounds due to their double lipid membrane, in this case, the greater efficacy against *Salmonella* could be attributed to eugenol's lipophilic nature and its ability to integrate into cell membranes, especially when encapsulated in nanoemulsions, which favor its penetration. Furthermore, factors such as the membrane composition, the presence of intracellular antioxidant defen-

se systems, or differences in efflux pump expression could influence the observed differential sensitivity. These results are consistent with some studies reporting high effectiveness of phenolic compounds against certain Gram-negative strains under specific conditions (Leyva-Jiménez *et al.*, 2019).

Eugenol primarily acts by disrupting the cell membrane, causing the leakage of essential intracellular components, including proteins and nucleic acids. The superiority of nanoemulsified eugenol over its free form is attributed to improved aqueous dispersion, stability, and bioavailability, which facilitate its interaction with bacterial cell membranes, leading to structural and functional alterations that result in microbial inactivation (Hu *et al.*, 2016). Furthermore, nanoemulsions enhance the stability of eugenol under environmental conditions (such as oxidation and volatility) and enable controlled release, thereby prolonging its antimicrobial effect during storage (Pernin *et al.*, 2019).

The findings of this study are consistent with previous research, which demonstrates the antimicrobial efficacy of eugenol encapsulated in nanoemulsions. For example, Hu *et al.* (2016) developed eugenol nanoemulsions using gum arabic and lecithin as emulsifiers, observing that the minimum inhibitory (750-1870 ppm) and bactericidal (900-3750 ppm) concentrations values of eugenol nanoemulsions were comparable to those of free eugenol (700-1500 ppm and 900-1870 ppm, respectively) across all tested strains of *L. monocytogenes* and *S. Enteritidis*, indicating that nanoemulsification did not diminish the intrinsic antibacterial activity of eugenol.

Similarly, Devi *et al.* (2025) reported that eugenol nanoemulsions exhibited inhibition zones of 18–23.5 mm against foodborne pathogenic bacteria, highlighting its potential as a natural preservative.

Reduction of Bacterial Load in Fresh-Cut Papaya Treated with Eugenol Nanoemulsion

The efficacy of eugenol-based nanoemulsions in reducing microbial load in fresh-cut papaya was evaluated by monitoring viable populations of *L. monocytogenes* and *S. Typhimurium* during 15 days of storage at 4°C. The treatments compared included a control containing bacteria exposed only to water, a blank (Tween 80 + water), and the application of nanoemulsions with different concentrations of eugenol (0.4 and 0.6 %).

As shown in Figure 2, on day 0, all treatments had an initial *L. monocytogenes* load of approximately 6 log CFU/g, as the samples were inoculated under controlled conditions. From day 5 onward, marked differences in bacterial load evolution were observed between treatments. *L. monocytogenes* populations increased in the control and blank treatments (Tween 80 + water), reaching levels of approximately 7.5 and 7.2 Log CFU/g at day 15, respectively, with no significant changes between them. This demonstrates that, in the absence of an active antimicrobial agent, fresh papaya provides a favorable environment for the proliferation of this pathogen, even when refrigerated. In contrast, treatments with nanoemulsions showed marked inhibition of bacterial growth. The 0.4 % eugenol nanoemulsion maintained populations below 6.5 Log CFU/g throughout storage. In comparison, the 0.6 % eugenol nanoemulsion was most effective, with a significant

decrease from day 1 (6.0 Log CFU/g) to a minimum of 4.4 Log CFU/g on day 5, followed by a slight recovery, but remaining below 5.5 Log CFU/g until day 15. These results confirm the prolonged bacteriostatic effect of the eugenol-enriched formulation.

Likewise, all fresh-cut papaya treatments on day 0 began with an *S. Typhimurium* load of approximately 6 log CFU/g, as the samples were inoculated in a controlled manner (Figure 3). *S. Typhimurium* populations showed sustained growth in the treatments without eugenol. The control and blank (Tween 80 + water) reached concentrations greater than 7.0 Log CFU/g at the end of storage. In contrast, treatment with a 0.4 % eugenol nanoemulsion stabilized the microbial load below 6.0 Log CFU/g, whereas the nanoemulsion with a higher eugenol concentration (0.6 %) induced a decrease from the start of the trial, reaching a minimum of 3.8 Log CFU/g on day 15. It is worth noting that the inhibitory effect of the nanoemulsion was more pronounced against *Salmonella* than against *Listeria*, consistent with observations in the *in vitro* test and likely due to the greater susceptibility of Gram-negative bacteria to phenolic compounds such as eugenol.

The results demonstrate that incorporating eugenol-containing nanoemulsions is an effective strategy for reducing bacterial load in FCFs, particularly papaya, which is highly perishable due to its high water activity and sugar content. The formulation containing 0.6 % eugenol exhibited prolonged bactericidal activity during refrigerated storage, likely due to the nanoemulsions' ability to protect and sustainably release the active compound from the fruit surface. This behavior can be attributed to the high bioavailability of eugenol in the colloidal system and its direct interaction with the microbial surface, which can alter the cell membrane,

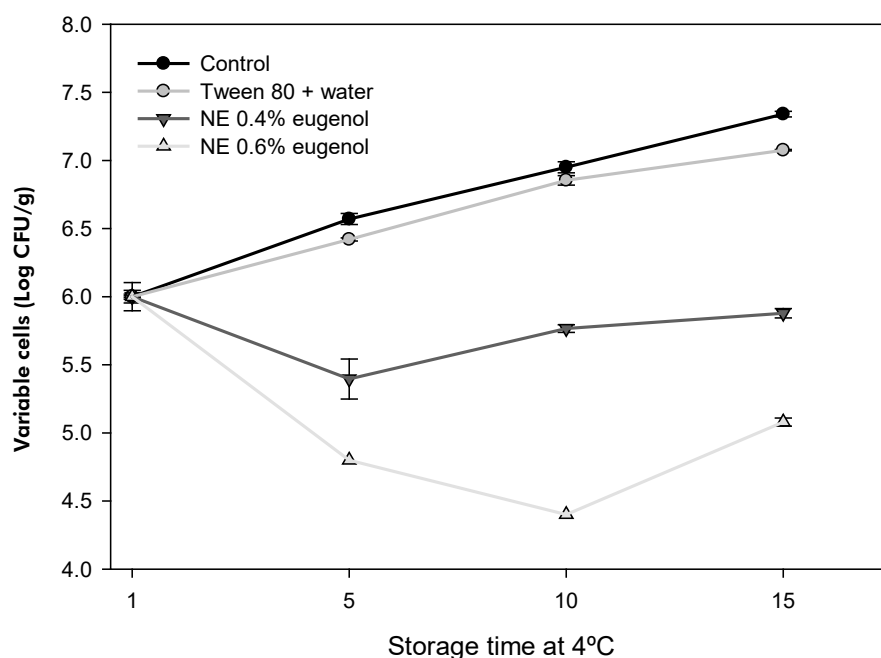


Figure 2. Effect of nanoemulsions (NE) with different concentrations of eugenol on the viability of *Listeria monocytogenes* in fresh-cut papaya during storage at 4°C.

Figura 2. Efecto de nanoemulsiones (NE) con diferentes concentraciones de eugenol sobre la viabilidad de *Listeria monocytogenes* en papaya fresca cortada durante el almacenamiento a 4°C.

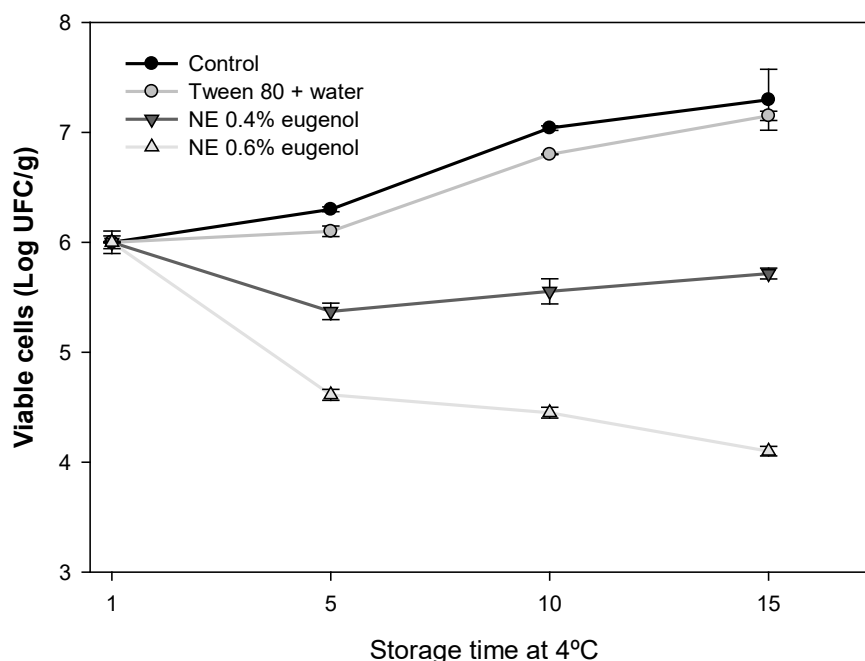


Figure 3. Effect of nanoemulsions (NE) with different concentrations of eugenol on the viability of *Salmonella* Typhimurium in fresh-cut papaya during storage at 4 °C.

Figura 3. Efecto de nanoemulsiones (NE) con diferentes concentraciones de eugenol sobre la viabilidad de *Salmonella* Typhimurium en papaya fresca cortada durante el almacenamiento a 4 °C.

lead to leakage of intracellular contents, and destabilize metabolism (Hasan *et al.*, 2019; Li *et al.*, 2022).

The results of this study confirm the efficacy of the eugenol-based nanoemulsion as an antimicrobial agent against *L. monocytogenes* and *S. Typhimurium* in FCFs. These findings are consistent with previous research highlighting the use of nanoemulsions in matrices with high water activity, such as papaya, melon, and mango, where moisture favors microbial growth. In these fruits, nanoemulsions formulated with eugenol have shown a remarkable ability to reduce microbial loads of relevant pathogens, including *E. coli*, *S. enterica*, and *L. monocytogenes*, without negatively affecting the color, taste, or texture of the product (Luciano *et al.*, 2023). A key aspect supporting its application is the possibility of using reduced concentrations of eugenol, enabled by its encapsulation, which prevents unwanted organoleptic alterations and preserves quality attributes such as firmness and color during refrigerated storage (Prakasha *et al.*, 2025). This effect has also been documented in other fruits, as shown in a study by Chen *et al.* (2024), in which the application of a lemon essential oil nanoemulsion to kiwifruit reduced the microbial load and slowed the decay rate without affecting fruit firmness.

Similarly, Touayar *et al.* (2023) developed citral and lemongrass essential oil nanoemulsions incorporated into alginate coatings applied to fresh-cut apples, demonstrating high system stability and a significant reduction in *L. monocytogenes* and *E. coli* over a three-week storage period. In the case of melon, Alencar *et al.* (2023) reported a 5 Log CFU/g reduction of *L. monocytogenes* using a citral nanoemulsion (15 µL/mL), reinforcing the potential of citral and other na-

noencapsulated phenolic compounds as viable preservation strategies for minimally processed fruits.

In addition to their individual efficacy, nanoemulsions have been proposed to enable the development of synergistic formulations. In this context, the results of the present study provide further evidence of the potential of eugenol nanoemulsion as an effective tool for preserving fresh fruits, particularly for ready-to-eat products that require natural and safe solutions to extend shelf life.

Sensory Evaluation of Fresh-Cut Papaya Treated with Eugenol Nanoemulsion

The sensory impact of nanoemulsions formulated with eugenol at different concentrations (0.4 % and 0.6 %) on fresh-cut papaya was evaluated, compared to an untreated and a blank treatment containing Tween 80 and water. The sensory attributes evaluated for papayas were aroma, taste, color, texture, and overall acceptability, using a 9-point hedonic scale. The results are presented in Figure 4.

Regarding aroma (Figure 4A), a significant decrease ($p \leq 0.05$) in the acceptance score was observed as the eugenol concentration increased. The control group obtained the highest average score (7.7), followed by the Tween 80 + water treatment (7.2). The 0.4 % eugenol nanoemulsion showed a reduction (4.9), while the 0.6 % eugenol nanoemulsion had the lowest acceptance (4.0), indicating that the characteristic aroma of eugenol may have been perceived as invasive or unpleasant by the evaluators. The decrease in acceptance could be attributed to eugenol's characteristic intensity, which at high concentrations can be invasive or unpleasant, masking the fruit's natural, fresh aroma. These results are

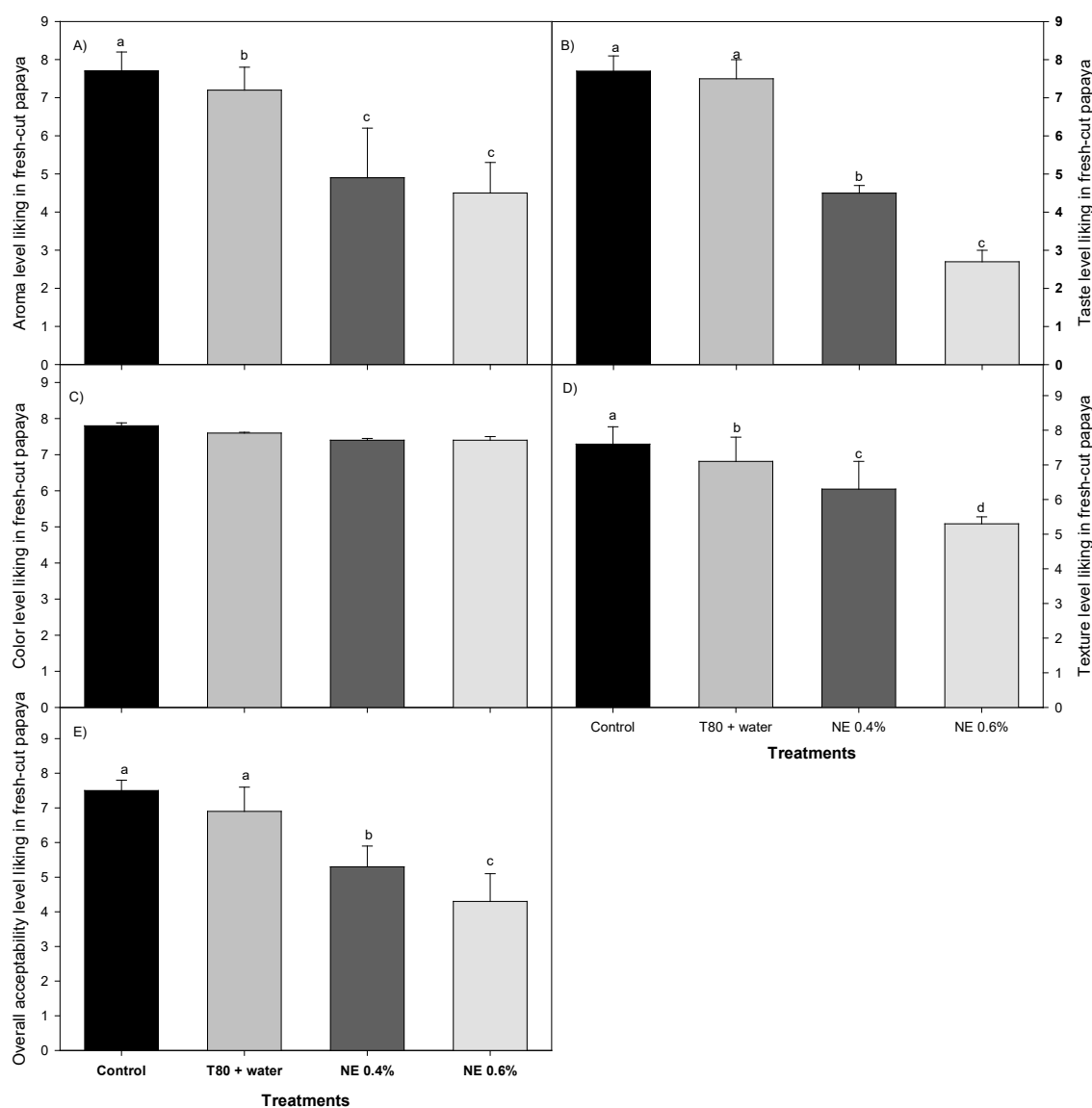


Figure 4. Sensory evaluation of A) aroma, B) taste, C) color, D) texture, and E) overall acceptability attributes of fresh-cut papaya treated with eugenol nanoemulsions (NE) at different concentrations. Different letters above the bars indicate significant differences between treatments ($p \leq 0.05$).

Figura 4. Evaluación sensorial de A) aroma, B) sabor, C) color, D) textura y E) aceptabilidad general de papaya recién cortada tratada con nanoemulsiones de eugenol (NE) a diferentes concentraciones. Diferentes letras indican diferencias significativas entre los tratamientos ($p \leq 0.05$).

consistent with previous studies, which indicate that the use of poorly dosed essential oils in fresh fruit can interfere with sensory acceptance, particularly in attributes such as smell and taste (Bleoancă *et al.*, 2025).

Among the sensory attributes evaluated, taste showed the greatest variability in scores and the greatest sensitivity to eugenol addition (Figure 4B). The control sample was the highest rated by the panel, with an average score of 7.7, closely followed by the blank sample (Tween 80 + water), indicating that the papaya in its natural state, as well as the one coated with the eugenol-free nanoemulsion, maintained an acceptable and pleasing taste profile for the evaluators. In contrast, the eugenol samples exhibited a noticeable decrease in taste scores. The 0.4% concentration was rated 5.0, while the 0.6% concentration reached only 3.2, which represents a

level close to rejection. This marked drop suggests that eugenol, although useful from an antimicrobial perspective, can significantly alter the fruit's taste profile, particularly at high doses. Eugenol has a characteristic, intense, and spicy taste commonly associated with cloves and, in some cases, can produce a mild bitterness or a burning sensation in sensitive mucous membranes. These properties, although beneficial in specific culinary contexts, are not always well-received when present in fresh fruits whose original taste is mild, sweet, and fruity, as is the case with papaya. Furthermore, its direct release into the mouth can result in an intrusive or discordant perception of the food's natural taste. Additionally, the possible interaction of eugenol with volatile compounds or sugars inherent to the fruit may alter the taste experience.

Regarding the color attribute, all treatments received

high ratings, with average scores above 7.3, indicating good overall visual acceptance of the samples and no impact of the eugenol nanoemulsion treatments (Figure 4C). The control sample obtained the highest score (7.8), followed by the blank sample (7.6), both of which had a fresh, characteristic appearance like that of freshly cut papaya. Meanwhile, the samples treated with eugenol nanoemulsions showed slightly lower scores, 7.4 for both 0.4 % and 0.6 %. Although these values remained within the acceptable range, they suggest a subtle influence of the coating on the product's visual perception. However, the difference was not significant enough to warrant outright rejection by the panel. It is essential to note that color is one of the most substantial and influential attributes in a consumer's purchasing decision. The fact that even the samples containing eugenol maintained a high score indicates that the nanoemulsions did not perceptibly alter the natural tone or appearance of the papaya, which is a positive result from a commercial and formulation perspective.

Texture is a fundamental sensory attribute in fresh produce, as it directly influences consumers' perception of quality and freshness. In this evaluation, the control sample obtained the highest average score (7.6), closely followed by the blank sample (7.1), suggesting that the base coating did not negatively affect papaya firmness or mouthfeel (Figure 4D). However, when eugenol was incorporated into nanoemulsions, a decrease in texture acceptance was observed. The 0.4 % sample scored 6.3, while the 0.6 % sample dropped to 5.2. This trend could be due to a slight modification in the fruit's surface structure caused by the coating, or to an altered tactile perception resulting from prolonged contact with eugenol. While the differences were not drastic, the progressive decrease in scores suggests that using higher concentrations of eugenol may cause some loss of firmness or interfere with papaya's characteristic juicy sensation. This response may be attributed to interactions between the phenolic compound and fruit cellular components, such as pectin, or to mild dehydration.

Overall acceptability summarizes evaluators' perceptions of a product, integrating attributes such as color, texture, odor, and taste. In this evaluation, the control sample obtained the highest score (7.5), followed by the blank sample (6.9), indicating that papaya, both in its natural state and the one coated solely with Tween 80 base, were well received by the panelists (Figure 4E). On the other hand, the eugenol-treated samples showed a notable decrease in overall acceptance. The 0.4 % formulation scored 5.3, while the 0.6 % sample dropped to 4.4, representing a significant reduction compared to the controls. This trend reflects the cumulative effect of eugenol on the various sensory attributes evaluated. While eugenol coatings offer advantages from a microbiological perspective, their impact on sensory perception cannot be overlooked. The low ratings for taste and odor decisively influenced the overall evaluation. Although color and texture were not severely affected, they were insufficient to offset the negative impact of the other attributes.

This result highlights the need to balance preservative functionality with the consumer's sensory experience. Overall acceptability is not only a preference metric but also a predictor of the product's commercial success. Therefore, if eugenol-containing nanoemulsions are to be applied to minimally processed fresh fruits, it will be essential to optimize their concentration, application method, and carrier matrix to ensure that their efficacy does not compromise consumer acceptance.

CONCLUSIONS

The results obtained throughout this work demonstrate that nanoemulsions formulated with eugenol represent a promising strategy for the microbiological preservation of minimally processed fresh fruits, such as papaya. Synthesis by direct sonication, combined with the use of a safe food surfactant, enabled the generation of stable colloidal systems with nanometric particle sizes, adequate surface charges, and high resistance to phase separation. From a microbiological perspective, nanoemulsions exhibited a significant antimicrobial effect both under *in vitro* conditions and when applied directly to food, effectively controlling the growth of relevant pathogens, such as *L. monocytogenes* and *S. Typhimurium*. This supports their functionality as food preservation agents without the need for synthetic additives. However, the sensory results showed that high concentrations of eugenol can negatively affect key attributes, such as product taste and odor, underscoring the importance of carefully adjusting formulations to balance microbiological efficacy and consumer acceptance.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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