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Effect of adding *Lippia origanoides* nanoemulsion to carboxymethyl cellulose films for active food packaging

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ABSTRACT

The food industry demands innovative packaging technologies to prolong shelf life and safeguard products from deterioration caused by ultraviolet radiation and microbial contamination. A promising strategy involves active packaging systems incorporating essential oil nanoemulsions, recognized for their antioxidant and antimicrobial efficacy. In this work, the physicochemical and biological attributes of an active film formulated with a nanoemulsion of *Lippia origanoides* essential oil (NE-OELO) were investigated to address oxidation and microbial contamination challenges. NE-OELO was synthesized using high-energy ultrasonic dispersion, comprising 5% (w/w) OELO, Tween 80 at a 1:2 ratio to OELO, and ultrapure water. The nanoemulsion was evaluated for droplet size, polydispersity index, zeta potential, whiteness index, and stability over a 120-day period. Active films were produced via the casting method, with NE-OELO incorporated at 10%, 15%, and 20% concentrations into a matrix containing 5% carboxymethylcellulose (CMC) and 1.5% glycerol. These films were characterized for mechanical, optical, thermal, and structural properties. Antioxidant capacity was measured using DPPH and ABTS assays, while antimicrobial activity was assessed by vapor-phase diffusion against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*. The films demonstrated UV barrier performance, reducing light transmission within the 200–350 nm range and nearly completely blocking light at 280 nm in higher NE-OELO concentrations (formulations F15 and F20). Antioxidant activity increased in a dose-dependent manner (Film F20, 35.89 ± 0.62 $\mu\text{mol TE/g film}$), likely attributable to phenolic constituents such as carvacrol. Growth inhibition of *S. aureus* was observed, whereas no inhibitory effects were detected against the Gram-negative bacteria tested. Mechanical testing revealed a reduction in tensile strength but enhanced flexibility (elongation) in F10 and F15, while transparency remained satisfactory. Notably, thermal stability decreased with increasing NE-OELO concentration, indicating the films are best suited for packaging refrigerated or fresh foods not exposed to post-packaging thermal processing. In summary, the incorporation of NE-OELO into a CMC matrix resulted in active films with effective UV-blocking capability, high antioxidant activity, and selective antimicrobial action against *S. aureus*. This study underscores the potential of nanostructured essential oil systems for developing advanced active packaging

solutions that effectively preserve food quality while maintaining essential visual and handling properties.

Keywords: *Lippia origanoides*, Essential Oil, Nanoemulsion, Active Packaging, Carboxymethylcellulose, Antimicrobial, Antioxidant.

1. INTRODUCTION

The food industry faces increasingly complex challenges in its efforts to extend the shelf life of products while maintaining their sensory and nutritional quality. A prominent factor contributing to spoilage and potential food safety concerns is the exposure of food to ultraviolet (UV) radiation. This radiation triggers photochemical reactions that degrade critical components of food, such as lipids and proteins. As a result, foods can become rancid and develop unpleasant flavors, which negatively impacts both their nutritional value and shelf life [1,2]. Moreover, contamination by pathogenic microorganisms further compromises food quality and poses significant risks to consumer health [3].

Contamination by pathogenic microorganisms, particularly *Staphylococcus aureus*, is a serious threat to public health, especially when it involves strains that produce heat-stable enterotoxins [4,5]. These toxins can lead to acute food poisoning, which is marked by symptoms such as nausea, vomiting, diarrhea, and abdominal cramps [6].

In response to these issues, active packaging has emerged as an innovative solution to improve food quality, stability, and safety. Specifically, active packaging that incorporates nanoemulsions containing essential oils can provide additional advantages. These benefits include enhanced physicochemical stability, controlled release of bioactive compounds, and increased antimicrobial and antioxidant Properties [7]. Since essential oils are well-known for their biological properties, which encompass both antioxidant effects and broad-spectrum antimicrobial activity against pathogenic microorganisms [8].

Furthermore, nanoemulsions increase the surface area of active agents [9], helping to mitigate issues such as volatility, low solubility, and undesirable sensory effects typically associated with the direct application of essential oils.

In this context, the genus *Lippia* holds significant interest, especially chemotypes rich in carvacrol, a monoterpene recognized for its antimicrobial properties, particularly its ability to disrupt bacterial cell membranes and inhibit

biofilm formation. Carvacrol is effective against pathogens such as *Staphylococcus aureus* [10-12].

A noteworthy study revealed that biodegradable films infused with thymol and carvacrol can effectively extend the shelf life of cheese [13]. These films, which contained 8% carvacrol and thymol, significantly reduced the microbial growth of pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*.

Among the species, *Lippia organoides* is particularly noteworthy for its chemotype, which comprises carvacrol as the predominant component of its essential oil [14]. This characteristic makes it a valuable raw material for the development of advanced packaging solutions. In this context, the present study aims to assess the physicochemical and biological properties of an active film incorporating a *Lippia organoides* essential oil nanoemulsion. This innovative approach is designed to address the challenges posed by oxidation and microbial contamination.

Among the natural polymers used to produce biodegradable films, carboxymethylcellulose (CMC) stands out as a cellulose-derived polysaccharide. CMC is highly soluble in water, tasteless and odorless, non-toxic, and has an excellent capacity to form stable matrices [15]. This makes it particularly effective for incorporating bioactive compounds, such as natural pigments, while also serving as a barrier against environmental factors. This contributes significantly to food protection and shelf-life extension [16].

In this context, the present study aimed to assess the thermal, physical, and physicochemical properties of CMC-based biodegradable films, as well as their antioxidant and antimicrobial activities, particularly when enriched with a nanoemulsion of *Lippia organoides* essential oil. This innovative approach aims to address the challenges associated with food spoilage.

2. MATERIALS AND METHODS

The medicinal plant *Lippia organoides* was collected in May 2024 from Riachão do Jacuípe, Bahia, Brazil (coordinates: 11.75062° S, 39.48387° W). The collection occurred in the morning, between 10:00 and 11:30 a.m., under dry weather conditions. A specialist identified the botanical material based on the

unique morphological characteristics of its leaves and inflorescences. The species identification was conducted by Lamarck do Nascimento Galdino da Rocha, and the specimen has been deposited in the Herbarium Collection of the Department of Biology at the State University of Feira de Santana (UEFS) in Feira de Santana, Brazil, under voucher number HUEFS259034. Additionally, access to the Brazilian genetic heritage has been registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) with the registration code A4F716D.

The essential oil was extracted by hydrodistillation using approximately 200 grams of dried plant material per extraction, yielding 2.13%. The oil was then stored in amber vials at $-20\text{ }^{\circ}\text{C}$ until needed; for preparing the nanoemulsion and films, Tween 80, carboxymethylcellulose, glycerol, and either ultrapure or distilled water were used, depending on the experimental phase.

2.1 Preparation of the Nanoemulsion

The nanoemulsion of *Lippia origanoides* essential oil (NE-OELO) was synthesized utilizing high-energy ultrasonic technology. This formulation comprised a 5% (w/w) concentration of essential oil, combined with a calculated 1:2 (w/w) ratio of essential oil to the non-ionic surfactant Tween 80, all dissolved in ultrapure water. The overall preparation process was executed in three distinct yet integral steps.

The nanoemulsion formulation was refined based on preliminary tests that helped identify the optimal concentration of the oil extract and the oil-to-surfactant ratio. These adjustments aimed to achieve a nanoemulsion that is both physically stable and compatible with application in films designed for active packaging production. The preparation process involved three distinct yet interrelated stages.

Initially, Tween 80 was thoroughly dissolved in ultrapure water and subjected to stirring at a speed of 1000 rpm for 10 minutes at ambient room temperature. This stirring was critical in ensuring a homogeneous dispersion of the surfactant in the aqueous medium. Following this, the essential oil was gradually added to the mixture, ensuring an even distribution throughout the solution. The resulting blend was then subjected to intensive processing using a

high-speed homogenizer, operating at 10,000 rpm for another 10 minutes. This step generated a stable precursor emulsion [15].

Subsequently, the precursor emulsion underwent a series of sonication cycles, specifically six sequential cycles of 5 minutes each. To maintain temperature stability and prevent overheating, the emulsion was kept in an ice bath throughout this process. This sonication was performed using ultrasonic apparatus (Ultronique – Eco-Sonics), which featured a 4 mm diameter probe functioning at a frequency of 20 kHz, with a power output of 550 watts. This ultrasonic treatment enhanced the emulsification process, yielding a finely dispersed nanoemulsion. Finally, the developed nanoemulsion was subjected to comprehensive characterization to assess its properties and performance.

2.2 Characterization of the Nanoemulsion

The characterization of the NE-OELO was conducted through various parameters, including droplet size, polydispersity index (PDI), Zeta potential (ZP), and the whiteness index (WI). To determine the droplet size, measured in nanometers (nm), along with the PDI expressed as a percentage (%), and Zeta potential quantified in millivolts (mV), the Zetasizer Nano ZS from Malvern Instruments (UK) was employed. To ensure accurate measurements and mitigate the impact of multiple scattering effects, the nanoemulsions were diluted with deionized water at a 1:100 ratio. All measurements were performed in triplicate to validate the data and ensure reproducibility.

To evaluate the stability of the nanoemulsion over time, the aforementioned parameters—droplet size, PDI, and zeta potential—were reassessed after 20, 40, and 120 days of storage under refrigerated conditions, to assess the physical stability of the formulations.

Additionally, the color depth of the nanoemulsion was measured using a colorimeter (CR-400, Konica Minolta) that was calibrated with the standard illuminant D65 to ensure consistency and accuracy. The color analysis utilized the CIE $L^*a^*b^*$ system, established by the Commission Internationale de l'Éclairage (CIE). In this framework, L^* represents lightness, while a^* and b^* correspond to redness and yellowness, respectively. Additionally, the whiteness index (WI) was calculated in accordance with Equation 1. [16]. This assessment

of color properties further contributed to the understanding of the nanoemulsion's overall quality and stability.

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b)^2} \text{ (Equation 1)}$$

2.3 Production of active films

The active films were produced through a precise casting process. To create the film-forming solutions, a blend of 5% carboxymethylcellulose (CMC) and 1.5% glycerol was dissolved in distilled water, resulting in a viscous and homogeneous mixture. To this base, varying concentrations of NE-OELO (10%, 15%, and 20% relative to the weight of the film-forming solution) were incorporated. These prepared solutions were then poured onto a glass plate, measuring 20 x 15 x 3 cm, forming a smooth, even layer. The films were dried under controlled conditions at 35 °C for 18 hours, ensuring optimal moisture removal and maintaining their structural integrity. Additionally, a control film without any nanoemulsion was simultaneously prepared for comparative purposes. Once dried, the films were sealed in plastic containers and stored at refrigeration temperatures to maintain quality until needed.

2.4 Characterization of active films

2.4.1 Determination of Thickness

The determination of film thickness is a critical parameter in characterizing active films, as it can significantly influence their physical properties and performance. In this study, the thickness of each film sample was measured using a micrometer (Mitutoyo). For each film sample, thickness measurements were conducted at three distinct locations across the surface to ensure a representative assessment of the film's uniformity. This method minimizes the potential impact of localized thickness variations that may arise during film fabrication. The average thickness was calculated by summing the individual readings and dividing by the total number of measurements. This method provides a more accurate representation of the film's characteristics.

2.4.2 Evaluation of Mechanical Properties

The mechanical properties of the films were assessed through a tensile test conducted with a TA.XT Plus texture analyzer, equipped with a 50 kg load cell. The testing was conducted under controlled conditions, with a pull speed of 5 mm/min, which ensured consistent strain application. The separation distance between the grips was maintained at 50 mm, ensuring accurate measurement of elongation.

For the evaluation, each film sample was prepared to dimensions of 20 mm × 100 mm, adhering to the standards [17]. A total of eight test samples were analyzed for each film type, providing a dataset for determining mechanical performance and ensuring reliability in the results.

2.4.3 Determination of Water Vapor Permeability

The water vapor permeability of the films was evaluated using the desiccant method [18]. In this procedure, film samples were cut into uniform squares and sealed with petroleum jelly inside acrylic capsules, which contained anhydrous calcium chloride to serve as the desiccant. These permeation cells were then placed in a controlled chamber where a saturated sodium chloride solution sustained the environmental conditions.

Over the course of the experiment, daily records of the chamber's temperature and relative humidity were taken, with the averages maintained at 24.7°C and 61%, respectively. To monitor the performance of the desiccant, the mass gain of the system was recorded through systematic weighings conducted at 2-hour intervals until the desiccant reached a mass gain threshold of 10%.

The data collected, which comprised mass gain over time, were plotted on graphs to facilitate the calculation of daily water permeation through the films, enabling the determination of the water vapor permeability (WVP) of the films, expressed in the unit of $\text{g}\cdot\text{mm}/\text{m}^2\cdot\text{day}^{-1}\cdot\text{kPa}$. To ensure the reliability and accuracy of these results, each measurement was performed in triplicate.

2.4.4 Optical Properties

The optical characteristics of the films were assessed using a CR400 colorimeter from Konica Minolta. To ensure a comprehensive evaluation, measurements were taken from each film at four distinct positions, following the

guidelines under standard illuminant D65, which closely simulates natural daylight.

The color of the films was evaluated using a CR400 colorimeter (Konica Minolta) under standard illuminant D65. Measurements were taken at four points on each sample, and the results were represented in the CIE L*a*b* system. In this system, L* indicates lightness (where 0 corresponds to black and 100 to white), a* reflects the variation between green (-a*) and red (+a*), and b* denotes the variation between blue (-b*) and yellow (+b*). The total color variation (ΔE), the yellowness index (YI), and the whiteness index (WI) were calculated using Equations 2, 3, and 4 [16]. Thereby enabling a thorough understanding of the films' color properties and visual appeal.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (\text{Equation 2})$$

$$YI = 142,86 \times b^*/L^* \quad (\text{Equation 3})$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad (\text{Equation 4})$$

2.4.5 Opacity and Light Transmission

The evaluation of opacity was conducted, utilizing a K37-UVVIS spectrophotometer from KASVI [19]. The films, trimmed to dimensions of approximately 3 x 1 cm, were positioned within a cuvette to ensure optimal measurement. For this assessment, an incident wavelength of 600 nm was selected. The opacity of the films was quantitatively calculated using Equation 5, providing a clear insight into their light-blocking properties.

In addition to opacity, the percentage of light transmission through the prepared active films was measured using a UV-Vis spectrophotometer (K37-UVVIS, KASVI). This measurement was conducted across a broad incident wavelength range of 200 to 800 nm [20]. This comprehensive approach enabled an analysis of how effectively the films allow light to pass through, thereby contributing to a better understanding of their optical properties.

$$Opacity = \frac{A_{600}}{E}$$

A600 = absorbance at 600 nm; E = film thickness (mm)

2.4.6 Evaluation of Thermal Properties

The thermal properties of the films were assessed using thermogravimetric analysis (TGA) [21]. Each film sample, weighing approximately 8 mg, was heated from a starting temperature of 30°C to a maximum of 800°C. This heating occurred at a controlled rate of 10°C per minute in an inert nitrogen atmosphere to prevent any oxidative reactions. The resulting TGA and derivative thermogravimetry (DTG) curves provided valuable insights for the thorough interpretation of the thermal stability and degradation behavior of the films.

2.4.7 X-ray Diffraction (XRD)

The analysis of the X-ray diffraction (XRD) patterns was conducted using a D2 Phaser benchtop X-ray diffractometer (Bruker), operating at a wavelength of $\lambda = 1.54184 \text{ \AA}$. The instrument was set to a voltage of 30 V and a current of 10 mA to optimize X-ray emission [22].

During the procedure, the films under examination were positioned at the center of a quartz holder, ensuring uniform exposure to the X-ray beam. The samples were then placed within the diffractometer for an analysis along the diffraction line, spanning from 5° to 90°. This method allowed for the identification and characterization of the crystalline structures present in the films.

2.4.8 Antioxidant Tests (DPPH and ABTS)

This section details the evaluation of the antioxidant properties of the films by measuring their free radical scavenging activity using two methods: the DPPH and ABTS assays. The films were first cut into 10 cm² squares, allowing for consistent sampling. These pieces were then placed in amber vials containing 10 mL of distilled water to facilitate dilution and protect the samples from light exposure.

The ABTS radical cation scavenging capacity was performed by using 50 μL of the diluted film sample, combined with 2 mL of a prepared ABTS radical solution (concentration of 7 mM), which included the addition of 2.45 mM potassium persulfate to generate the radical [23]. Specifically. The reaction was

monitored continuously, capturing the dynamic changes in absorbance at 734 nm using a K37-UVVIS spectrophotometer (KASVI). A calibration curve was constructed using six Trolox standards with concentrations ranging from 40 to 400 mg. L⁻¹, allowing for accurate quantification of the antioxidant activity.

DPPH free radical scavenging assay was performed by adding 2.5 mL of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution to 0.5 mL of the diluted film samples. The reactions were conducted at room temperature, with protection from ambient light to prevent interference. The absorbance of the film/DPPH solutions and control blanks was measured at 517 nm, using a spectrophotometer. Calibration for this method was also established, using six Trolox standards within the concentration range of 10 to 160 mg. L⁻¹[24].

The resulting antioxidant activities of the films were quantified and expressed as micromoles of Trolox equivalent per gram of film (μmol Trolox eq/g of film), providing a clear indication of their efficacy in scavenging free radicals.

2.4.9 In Vitro Antimicrobial Activity of Volatile Compounds from the Active Film

In this study, the microorganisms *Staphylococcus aureus* (ATCC 00039), *Escherichia coli* ETEC (ATCC 11105), and *Salmonella typhimurium* (ATCC 14028) were initially activated in brain heart infusion (BHI) broth and incubated at 37 °C for 18 to 20 hours, allowing for optimal growth conditions to occur. Following this incubation period, the microbial suspension was adjusted to a standardized concentration of approximately 1.5 × 10⁸ CFU.mL⁻¹, utilizing a spectrophotometer calibrated to an optical density (OD) range between 0.08 and 1 at a wavelength of 625 nm [25].

To evaluate the antimicrobial effects of the volatile compounds emanating from the active film impregnated with essential oil nanoemulsion, the vapor phase diffusion method was applied. The adjusted microbial suspension was spread uniformly across the surface of sterile Petri dishes filled with Mueller-Hinton Agar using a sterile swab [25, 26].

Subsequently, circular cutouts of the active film, each with a diameter of 10 cm, were made at varying concentrations of 0% (as a control), 10%, 15%, and 20%. These cutouts were subjected to UV sterilization for 30 minutes to eliminate

any potential contaminants. They were then positioned on the insides of the Petri dish lids using inert, UV-sterilized double-sided tape.

The Petri dishes, now containing the inoculated culture medium, were immediately sealed with the fitted lids, ensuring that the active film remained suspended above the agar surface to facilitate interaction with the volatile compounds. To further enhance the containment of these compounds and prevent any risk of contamination, the outer edges of each dish were hermetically sealed with Parafilm.

The sealed plates were incubated at 37 °C for 24 hours. After this incubation period, the plates were subjected to a thorough visual inspection to assess the extent of microbial growth, allowing for the evaluation of the antimicrobial efficacy of the volatile compounds under study.

2.4.10 FT-IR Analysis

The infrared spectrum of the films was examined using an FT-IR spectrometer (Agilent Cary 630 FTIR) within a wavenumber range of 4000 to 650 cm^{-1} . This analysis was performed with the aid of an attenuated total reflectance (ATR) module, which facilitated the collection of precise spectral data and provided valuable insights into the functional groups present in the films.

2.5 Statistical Analysis

The experimental procedures were performed in triplicate, following a completely randomized design to minimize bias. The resulting data underwent thorough analysis using Analysis of Variance (ANOVA) and Tukey's test, both conducted with a significance level of 5%. This comprehensive statistical approach, employed using Statistica software (version 12), ensured the evaluation of the results. All data are presented as the mean $\pm$ standard deviation (SD) to reflect the variability within the measurements accurately.

3 RESULTS AND DISCUSSION

3.1 Characterization of the *Lippia origanoides* Essential Oil Nanoemulsion

The nanoemulsion formulated from *Lippia organoides* essential oil demonstrated an average droplet size of 59.7 ± 0.99 nm, which falls within the nanometric range of 20–200 nm [27]. The smaller droplet sizes observed in this study are significant, as they minimize the tendency for droplets to settle under the influence of gravity and improve the dispersion of the lipophilic compound within the continuous phase [28].

This value is consistent with the properties of nanoemulsion systems designed for incorporation into polymer matrices. Droplets in this size range have a high interfacial area, which enhances interaction between bioactive compounds and the film-forming matrix. Additionally, these droplets help to mitigate gravitational effects such as sedimentation and creaming, while improving the dispersion of lipophilic compounds within the continuous phase [30]. Consequently, the dispersion is expected to be more physically stable, with a more uniform distribution of the oil phase during film formation.

The results obtained align with findings from other studies that employed essential oils and ultrasonication. For example, a study [29] reported average droplet sizes of around 50 nm for nanoemulsions created from oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and lemongrass (*Cymbopogon citratus*) essential oils. The small droplet diameter, along with the monomodal distribution, can be attributed to the efficacy of the ultrasound treatment in conjunction with the surfactant Tween 80. The shock waves generated by ultrasonic cavitation enhance the breaking and mixing of phases, transforming larger droplets into smaller ones [29]. Furthermore, both the duration of the ultrasound treatment and the oil-to-surfactant ratio play significant roles in determining the size of the nanoemulsion droplets [30].

The polydispersity index (PDI) of NE-OELO was determined to be 0.1365 ± 0.0254 . This low value, falling below 0.3, indicates a uniform particle size distribution, signifying a monodisperse system. Such homogeneity is highly advantageous as it minimizes the formation of droplets with diverse and extreme sizes, thereby enhancing the overall stability of the system [31].

This characteristic is essential for active packaging applications, as narrow distributions facilitate the uniform incorporation of the oil phase into the carboxymethylcellulose matrix. This uniformity minimizes localized concentration

regions, which can compromise the material's mechanical, optical, and barrier Properties [33].

The measured zeta potential was -17.89 ± 1.31 mV. This measurement quantifies the effective electrostatic charge at the droplet interface and serves as a key parameter for assessing the electrokinetic behavior and stability of colloidal dispersions. It influences various phenomena, including sedimentation, agglomeration, and particle deposition. Furthermore, the zeta potential is influenced by several factors, including the pH of the medium, the ionic strength of the solution, the particle's physicochemical properties, and the system's temperature [32].

The zeta potential value observed in this study suggests a moderate level of electrostatic stability, likely enhanced by the steric stabilization provided by the surfactant Tween 80. This combination of electrostatic repulsion and steric stabilization plays a crucial role in the overall stability of the formulation. Similar findings have been reported in other formulations, such as the *Nigella sativa* oil nanoemulsion, which exhibited a zeta potential of -14.17 mV [33].

Although this result does not necessarily imply low stability, it is essential to recognize that in systems stabilized by non-ionic surfactants such as Tween 80, stability is primarily governed by the steric mechanism. The surfactant's hydrophilic chains form a protective barrier around the droplets, minimizing their tendency to merge and restricting processes such as flocculation and coalescence, even in the presence of moderate electrostatic repulsion [34].

In summary, droplet size parameters of less than 200 nm, a low polydispersity index (PDI) of under 0.3, and a negative zeta potential suggest that the nanoemulsion exhibits a monomodal, homogeneous, and stable distribution. These results are consistent with recent advancements in nanoemulsion formulations of essential oils, emphasizing that well-controlled emulsification conditions and the careful selection of surfactant combinations can significantly enhance the long-term stability of nanoemulsions [34].

The combination of reduced droplet size, low polydispersity, and steric stabilization enhances dispersion stability while directly influencing the final performance of the films. This interplay is particularly relevant to key characteristics, including transparency, antioxidant activity, and the uniform distribution of bioactive compounds throughout the polymer matrix [36].

3.2 Stability of Nanoemulsion During Storage

The physicochemical stability of the nanoemulsion throughout the storage period is a crucial parameter, as it reflects the system's ability to maintain its nanometric characteristics and, consequently, its biological and technological effectiveness. Figure 1 illustrates the changes in average droplet size (measured in nanometers) and the Polydispersity Index (PDI) of NE-OELO over the course of storage (in days).

The physicochemical stability of nanoemulsions plays a pivotal role in their application within active packaging systems. Maintaining consistent droplet size and homogeneous dispersion directly influences the distribution of bioactive compounds within the polymer matrix, their release kinetics, and, consequently, the overall functional effectiveness of the developed material.

The particle size, illustrated in Figure 1A, exhibited a progressive increase over the 120-day analysis period. Initially, the droplet size was approximately 60 nm. By the end of the 120 days, the average droplet size had increased to approximately 155 nm. Although there was an increase in droplet diameter, the size remained significantly below the 200 nm threshold, which is typically considered indicative of a stable nanoemulsion system for essential oils.

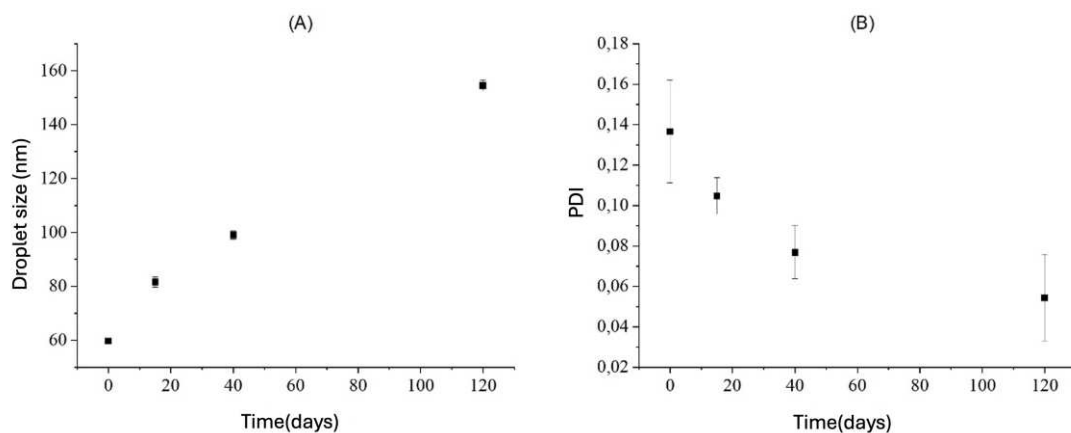


Fig. 1 Droplet size and polydispersity of *Lippia origanoides* essential oil nanoemulsion over storage time.

The growth observed, resulting from phenomena such as flocculation and subsequent coalescence, suggests that stability is maintained within acceptable limits. However, this factor is important to consider when evaluating the

formulation's shelf life. Maintaining the system below 200 nm for an extended period demonstrates good physical stability of the nanoemulsion throughout storage. This stability is essential, as an increase in particle size can lead to agglomeration and coalescence, which may compromise the nanoemulsion's effectiveness.

The observed growth can primarily be attributed to slow physical instability mechanisms, including partial coalescence, reversible flocculation, and Ostwald ripening. Among these, Ostwald ripening is particularly noteworthy, as it significantly contributes to the gradual increase in droplet size in nanoemulsions containing hydrophobic compounds. This phenomenon occurs because smaller droplets are more soluble, driving diffusion of the oil phase toward larger droplets over time. However, the slow growth rate suggests that Tween 80's effective interfacial stabilization considerably hindered this process [37].

The increase in the average droplet size may be related to the observed zeta potential value of -17.89 ± 1.31 mV. Zeta potential values with a magnitude less than ± 30 mV suggest moderate electrostatic stability, allowing particles to remain in proximity during storage [35,36]. This condition accounts for the gradual growth of the droplets, which remains within a range that ensures good physical stability of the nanoemulsion.

Although the initial zeta potential (-17.89 mV) indicates only moderate electrostatic stabilization, the maintenance of stability over 120 days shows that the nanoemulsion's primary stabilization mechanism was steric. The polyoxyethylene chains of Tween 80 adsorbed onto the droplet surfaces form a physical barrier that hinders droplet approach and minimizes coalescence, even under conditions where electrostatic repulsion alone would be insufficient to prevent aggregation [38,39].

The graph presented in Figure 1B illustrates the variations in the Polydispersity Index (PDI), a key measure of the uniformity within particle size distributions. The initial PDI value, approximately 0.14, suggests a narrow and homogeneous (monodisperse) distribution of particles. This characteristic is crucial for ensuring the system's efficiency.

The PDI remained consistently low, at less than 0.14, throughout the 120 days. This stability indicates that the system effectively prevented the formation of particle populations with extreme sizes, likely due to the partial and controlled

fusion of smaller droplets with larger ones. This process reduces size variability, resulting in a more homogeneous and stable system [28,37,38].

A particularly significant aspect was the ability to maintain low polydispersity index (PDI) values throughout storage. Contrary to what is usually expected in systems experiencing considerable instability, the PDI consistently remained below 0.14. This observation suggests that droplet growth occurred relatively uniformly, without the emergence of secondary populations of larger particles. This behavior indicates that the nanoemulsion reorganized in a controlled manner, preserving its homogeneity. This feature is especially critical for applications in biodegradable films, as it minimizes heterogeneous regions during drying of the film-forming solution and promotes a more uniform distribution of the oil phase within the polymer matrix. [30,40,41].

Maintaining the PDI within a range of 0.1 to 0.25 is highly desirable, as these values suggest a narrow particle distribution. This narrow distribution is a key factor that enhances both the stability and effectiveness of the system [39,40], according to a study that was reported a stable nanoemulsion formulation containing *Lippia origanoides* essential oil, with an average nanoparticle size of 167.5 nm and a PDI less than 0.2, maintaining its physicochemical stability and narrow particle size distribution [41].

Similar findings have been reported in studies on nanoemulsions containing essential oils stabilized by non-ionic surfactants. However, studies indicate significant increases in both mean size and polydispersity index after extended storage periods [42,43,44]. In the present study, although mean diameter increased, the size distribution remained largely unchanged. This outcome suggests that the combination of applied ultrasonic energy and the oil-to-surfactant ratio was highly effective, resulting in a stable interface that minimizes irreversible aggregation.

Figure 1 illustrates that NE-OELO maintains physical stability throughout a 120-day storage period. Although there was an increase in droplet size from 60 nm to 155 nm, the integrity of the nanometric system remained intact, while homogeneity was preserved with a consistently low polydispersity index (PDI). This stability is vital for future technological applications, as it ensures the nanoemulsion's integrity, thereby extending the release time of the bioactive

compounds from the essential oil. This characteristic is fundamental to the effectiveness of the active film.

From a technological standpoint, this stability has significant implications for active-film performance. Homogeneous nanoemulsions generally create films with a more uniform distribution of bioactive compounds, leading to fewer structural defects, reduced lipid aggregation, and more consistent mechanical, optical, and barrier properties. Furthermore, maintaining nanometric dimensions during storage helps to preserve the high surface area of the droplets, a factor that can improve antioxidant activity and promote the gradual release of phenolic compounds from the essential oil once it is incorporated into the film.

3.3 Mechanical Properties and Water Vapor Permeability

The measured values (Table 1) for water vapor permeability, tensile strength, and elongation illustrate the effects of incorporating the nanoemulsion into the films. This incorporation strikes a balance between barrier properties and flexibility, both of which are crucial for biotechnological applications and active packaging. The values for the carboxymethylcellulose (CMC)-based films, which include varying concentrations of NE-OELO, are presented in Table 1.

Water Vapor Permeability (WVP) is a parameter that evaluates a film's ability to inhibit moisture transfer, significantly impacting both food quality and shelf life [42]. The results indicate that the incorporation of NE-OELO affected the WVP, with films containing a 10% concentration of nanoemulsion exhibiting increased permeability compared to the control film.

Table 1 – Water vapor permeability and mechanical properties of carboxymethylcellulose films with different concentrations of *Lippia organoides* essential oil nanoemulsion.

Film	WVP (g.mm/m ² .dia.kPa)	MR (MPa)	Elongation (%)
F0	6,65 ± 0,33 c	22,59 ± 2,47 a	55,65 ± 0,32 b
F10	11,25 ± 0,59 a	12,66 ± 1,27 b	80,64 ± 8,50 a
F15	8,42 ± 0,50 b	15,25 ± 0,30 b	77,32 ± 5,99 a
F20	8,03 ± 0,35 b	15,44 ± 1,38 b	41,46 ± 3,28 b

*In the columns, means with the same letters do not differ statistically from each other, according to Tukey's test, at the 5% probability level. WVP– Water vapor

permeability; MR – Mechanical resistance. F0 Control film (0% NE-OELO); F10 (10% NE-OELO m/m); F15 (15% NE-OELO m/m); F20 (20% NE-OELO m/m).

This behavior can be attributed to the initial reorganization of the polymer network triggered by the introduction of dispersed lipid droplets. During film drying, these nanoparticles impede proximity between carboxymethyl cellulose (CMC) chains, decreasing hydrogen-bond density and increasing the free volume available for water-molecule diffusion.

Interestingly, formulations F15 and F20 show a distinctive behavior. Although their permeability remains higher than that of the control film, it is notably lower than that of F10. This finding suggests that higher nanoemulsion concentrations induce a specific reorganization of the film's microstructure. Rather than simply increasing the free space between polymer chains, more droplets promote the formation of more homogeneous lipid domains throughout the matrix.

These lipid domains increase the tortuosity of the path that water molecules must navigate, requiring vapor to circumvent hydrophobic regions before penetrating the film. As a result, while the matrix remains predominantly hydrophilic due to the CMC, the more uniform distribution of the oil phase partially limits vapor diffusion, explaining the improvements observed in formulations F15 and F20.

This behavior demonstrates that the relationship between nanoemulsion concentration and permeability is not linear. It stems from the interplay between two concurrent phenomena: increased free volume from initial matrix disruption and the progressive formation of hydrophobic barriers distributed within the film [46].

This interpretation aligns with recent studies on nanoemulsions in cellulosic matrices. However, unlike other studies that report a continuous increase in permeability with increasing lipid content, this study's results indicate that the nanoemulsion's high homogeneity ($PDI < 0.14$) facilitated uniform dispersion. This uniformity has minimized aggregate formation, allowing the hydrophobic regions to restrict water diffusion more effectively.

The observed increase in water vapor permeability (WVP) upon incorporating nanoemulsions into hydrophilic films, such as carboxymethyl

cellulose (CMC), is a well-documented phenomenon. The lipid nature of the essential oil, combined with surfactants such as Tween 80, disrupts the packing of the polymer network within the CMC matrix. This disruption results in tortuous pathways and pores that facilitate the diffusion of water molecules through the film [43].

The reduction in WVP observed in F15 and F20, when compared to F10, suggests that higher concentrations of NE-OELO may facilitate improved dispersion and void filling, or enhance the formation of interconnected lipid domains. These domains appear to restrict water passage more effectively than at lower concentrations, although they do not entirely overcome the barrier provided by pure CMC.

Similar findings were reported in a research on active films incorporating *Chenopodium ambrosioides* essential oil [44]. They noted that higher concentrations of the essential oil decreased water vapor permeability due to the development of interconnected lipid domains within the polymeric matrix.

A study examining active films containing *Chenopodium ambrosioides* essential oil reported comparable results. It was noted that higher oil concentrations contributed to the formation of hydrophobic regions dispersed within the polymer film. These regions increased the tortuosity of the water vapor diffusion path, reducing permeability, without forming a continuous lipid phase in the system [47].

When examining the mechanical properties, it was noted that the tensile strength of the films decreased with the incorporation of NE-OELO, with no significant differences found among the films that included this additive. This reduction in strength suggests that NE-OELO may disrupt the intermolecular interactions between polymer chains, resulting in increased flexibility, decreased mechanical strength, and enhanced water vapor permeability [45].

This reduction indicates that the dispersed lipid droplets function as physical discontinuities within the polymer film, disrupting intermolecular interactions, such as hydrogen bonds, between the carboxymethyl cellulose (CMC) chains. Even at low concentrations, these droplets can increase the spacing between polymer chains and diminish network cohesion. As a result, the film shows greater flexibility, reduced mechanical strength, and changes in water vapor permeability [48]. During film formation, the nanostructured droplets

occupy spaces between polymer chains, increasing intermolecular spacing and reducing the density of hydrogen bonds between the hydroxyl and carboxymethyl groups of CMC. This, in turn, promotes greater chain mobility and reduces the stress required for material rupture.

Similar findings were observed regarding the reduction in mechanical strength when developing CMC films infused with cinnamon essential oil (*Cinnamomum cassia*). The control film demonstrated a strength of 21.87 MPa, while the film containing 8% (w/v) of cinnamon oil exhibited a strength of only 13.55 Mpa [46].

Comparable results concerning the reduction in mechanical strength were noted during the development of CMC films infused with cinnamon essential oil *Cinnamomum cassia*. This observation is consistent with the findings of the current study and further supports the notion that incorporating lipid nanoemulsions acts as a physical plasticizer, thereby modifying the film's mechanical Properties [49].

A research demonstrated that CMC films exhibited a reduction in maximum tensile strength, decreasing from 24.1 MPa in the control film to 16.4 MPa in the film that incorporated the essential oil nanoemulsion. The authors attribute this change to the nanostructured dispersion, which results in increased heterogeneity and enhanced flexibility within the matrix [47].

Another study examined CMC films infused with nanoemulsions of various essential oils, including thyme, clove, and cardamom. They reported tensile strength values ranging from 11.7 to 20.4 MPa. Their research demonstrated that an increase in the lipid content derived from the essential oils in the film results in a decrease in the cohesive forces between the CMC chains. This behavior aligns with the observations made in the present study [48].

The increase in elongation seen in films containing 10% and 15% NE-OELO, compared to the control, is particularly noteworthy. This enhancement illustrates the plasticizing effect of the nanoemulsion, which imparts greater flexibility to the material and enhances its ability to deform without fracture [49]. These results highlight the role of nanoemulsions as rheological modifiers, enhancing the distribution and interaction of phases and thereby optimizing the flexibility of the films.

The increase in elongation in formulations F10 and F15 suggests that the nanoemulsion enhances polymer-chain mobility, enabling greater deformation before rupture. This behavior exemplifies a physical plasticizing effect, wherein the dispersed phase reduces intermolecular forces without inducing permanent chemical changes to the CMC structure [52]. This phenomenon underscores that the plasticizing effect induced by the nanoemulsion is closely linked to the spatial distribution of the nanoparticles. At low to medium concentrations, enhanced molecular mobility prevails; however, at higher concentrations, interactions between droplets can limit this mobility to some extent.

The F20 film exhibited an elongation statistically comparable to that of the control film (F0), yet it was significantly lower than that of the F10 and F15 films. This behavior can be attributed to the presence of excess particles within the polymer matrix. At high concentrations, these dispersed particles can serve as stress concentration points or antiplasticizing agents, thereby limiting chain mobility and impeding the film's deformation ability [50-52].

Similar results were demonstrated in a research on biodegradable methylcellulose films for packaging perishable foods [53]. The study reported a reduction in tensile strength accompanied by an increase in elongation, which they attributed to alterations in the internal organization of the polymer matrix.

In the evaluation of biopolymer films that incorporate cellulose nanocrystals and peppermint essential oil (*Mentha x piperita* L.), it was observed that the elongation values of the films increased significantly, ranging from 2.18% to 8.10%. Conversely, there was a decline in the mechanical strength of the films [54]. This observation indicates a correlation where higher elongation values correspond with lower tensile strengths, and conversely [55].

The incorporation of NE-OELO into the CMC matrix led to modifications in the polymeric structure, which were evident in the changes to the film properties. Although the water vapor barrier capacity was compromised by an increase in WVP, representing a drawback for packaging moisture-sensitive foods, a significant improvement in flexibility (elongation) was observed in concentrations F10 and F15. This enhancement is advantageous for handling. The alterations in the films' properties suggest their potential as functional layers in multilayer films. Such configurations could effectively balance the flexibility and biofunctional activity of the CMC and NE-OELO layer with the high moisture resistance

provided by a second hydrophobic layer, thereby expanding their applications in biodegradable food packaging.

3.4 Optical Properties

The optical properties of the films, especially their transparency, are essential in food packaging. These characteristics are important not only for ensuring food protection but also for influencing consumer purchasing choices, as many individuals prefer to see the food products they are buying [56-58].

Table 2 presents the results for opacity, total color variation (ΔE), the yellowing index (YI), and the whiteness index (WI) of carboxymethylcellulose (CMC) films incorporating various concentrations of *Lippia origanoides* essential oil nanoemulsion (NE-OELO).

Table 2 – Optical properties of carboxymethylcellulose films incorporated with different concentrations of *Lippia origanoides* essential oil nanoemulsion.

Film	Opacity (mm^{-1})	ΔE	YI	WI
F0	1,39 $\pm$ 0,29 b	2,45 $\pm$ 0,08 a	-3,72 $\pm$ 0,03 a	90,99 $\pm$ 0,05 a
F10	3,39 $\pm$ 0,27 a	2,40 $\pm$ 0,08 a	-3,98 $\pm$ 0,11 a	90,89 $\pm$ 0,10 a
F15	3,37 $\pm$ 0,45 a	2,39 $\pm$ 0,19 a	-3,83 $\pm$ 0,15 a	90,84 $\pm$ 0,10 a
F20	3,46 $\pm$ 0,39 a	2,35 $\pm$ 0,07 a	-3,79 $\pm$ 0,07 a	90,88 $\pm$ 0,09 a

*In the columns, means that share the same letters do not differ statistically from each other, according to Tukey's test, at the 5% probability level. ΔE indicates Total Color Variation; YI represents the Yellowing Index; WI denotes the Whiteness Index. F0 Control film (0% NE-OELO); F10 (10% NE-OELO m/m); F15 (15% NE-OELO m/m); F20 (20% NE-OELO m/m).

The analysis of the optical properties indicated that the films containing NE-OELO exhibited higher opacity compared to the control film. Additionally, the opacities of films F10, F15, and F20 demonstrated statistically significant differences from one another, as shown in Table 2. Films F10, F15, and F20 exhibited similar opacities that were significantly higher than the F0 control.

The other variables, ΔE , YI, and WI, showed no statistically significant differences and maintained stable values across the formulations. This finding indicates that the color and visual transparency of the films remained consistent. The results suggest that although the addition of the nanoemulsion causes slight

turbidity due to the uniform dispersion of NE-OELO, it does not compromise the appearance or optical uniformity of the polymeric matrix.

Thus, the results of the optical property tests on the films in this study align with the functional objective of protecting the packaged contents without compromising the film's transparency, as shown in figure 2.

The visual comparison of the films shown in figure 2 clearly illustrates their transparency, even at higher NE-OELO concentrations.

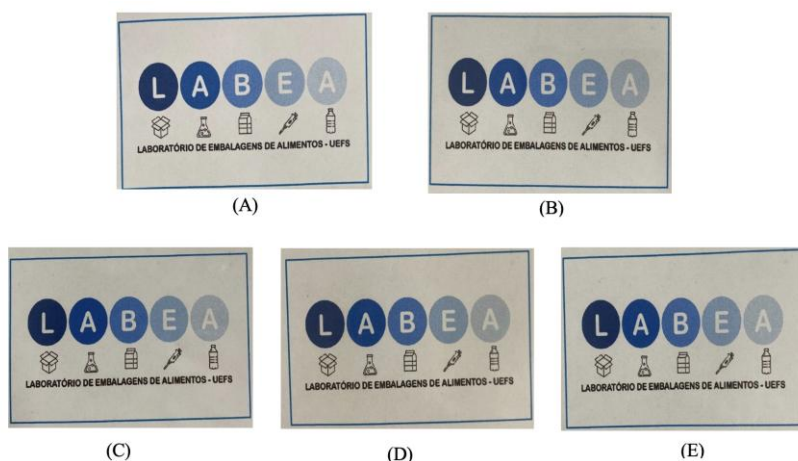


Fig. 2 Appearance of active films on the same visual background: (A) Image without film (B) Control film (0% NE-OELO) (C) Film 10 (10% NE-OELO m/m) (D) Film 15 (15% NE-OELO m/m) (E) Film 20 (20% NE-OELO m/m).

Biodegradable CMC films infused with *Thymus* essential oil revealed that all samples displayed opacity values ranging from 7.47 to 14.84 mm^{-1} , which were lower than those observed in the control samples. Conversely, the addition of essential oils (EOs) significantly enhanced the transparency values. This improvement indicates a favorable interaction between the EOs and the hydrophilic matrix of the polymer, promoting interfacial compatibility. As a result, the films formed very fine, uniformly distributed emulsions, which reduced light scattering and ultimately improved the overall transparency of the film [59].

CMC films enhanced with curcumin and natural pigments revealed that opacity values varied from 1.2 to 3.5 mm^{-1} , depending on the concentration of the active ingredient. The color and transparency characteristics remained statistically consistent across the various formulations, attributed to the uniform dispersion of the pigments and their effective interaction with the polymeric matrix. The authors underscored the potential of these films as materials for innovative packaging, noting their color stability and physical Properties [60].

Transparent films enhance the visual appraisal of food, improving consumers' perceptions of freshness and quality by allowing a clear view of the product inside. Additionally, the use of transparent active films emulates the appearance of traditional plastic packaging, increasing aesthetic appeal and expanding their applications in the food industry [54, 61,62].

While greater opacity may be seen as a marketing drawback in situations requiring high transparency, it provides significant benefits in packaging designed to protect against photodegradation. This includes safeguarding against lipid oxidation and vitamin degradation, which serves as a compelling marketing point for prolonging shelf life.

3.5 Light Transmission of Films

The capacity of packaging films to filter light radiation is essential for food protection, particularly against photo-oxidation and the breakdown of photosensitive compounds [63]. Table 3 illustrates the percentage of light transmission (%) for carboxymethylcellulose (CMC) films containing various concentrations of *Lippia origanoides* essential oil nanoemulsion (NE-OELO) across different wavelengths (in nanometers).

Table 3 – Light transmission of carboxymethylcellulose films containing various concentrations of *Lippia origanoides* essential oil nanoemulsion.

Fil m	Wavelength (nm)							
	200	280	350	400	500	600	700	800
F0	1,1 ± 0,5	55,9 ± 3,7	71,1 ± 1,6	77,3 ± 1,0	81,1 ± 0,7	82,8 ± 0,6	83,7 ± 0,7	84,5 ± 0,7
F1	0,3 ±	2,9 ±	60,9 ±	63,0 ±	65,6 ±	68,8 ±	70,0 ±	72,5 ±
0	0,4	1,5	6,4	6,2	6,0	5,3	4,7	4,0
F1	0,0 ±	0,4 ±	54,9 ±	60,3 ±	62,9 ±	64,6 ±	66,4 ±	68,3 ±
5	0,0	0,2	3,1	3,2	3,0	2,8	2,9	2,9
F2	0,0 ±	0,0 ±	55,3 ±	57,5 ±	58,5 ±	60,5 ±	62,6 ±	64,9 ±
0	0,0	0,0	4,9	6,2	6,0	5,6	5,5	5,3

Carboxymethylcellulose films incorporated with varying concentrations of *Lippia organoides* essential oil nanoemulsion: F0 (0% NE-OELO), F10 (10% NE-OELO), F15 (15% NE-OELO), and F20 (20% NE-OELO).

The control film (F0) exhibited high transmittance across the entire visible spectrum (400–800 nm), achieving nearly 85% at 800 nm, which demonstrates its transparency. In contrast, the incorporation of NE-OELO resulted in a gradual decrease in the transmittance of the films across the entire spectrum, particularly in the ultraviolet range (200–350 nm). This reduction was especially pronounced in formulations with higher concentrations of nanoemulsion (F15 and F20), indicating a significant capacity to block ultraviolet radiation.

The significant impact of NE-OELO concentration underscores the efficacy of *Lippia organoides* nanoemulsion films as optical barriers, particularly against ultraviolet radiation. This property is essential for active packaging designed to protect light-sensitive compounds and to mitigate oxidative reactions or nutrient loss in food, thereby preserving the organoleptic qualities of the packaged items [64]. The observed reduction in light transmission is attributed to the polyphenolic compounds present in the chemical composition of *Lippia organoides* essential oil [65].

Polymer-based films incorporating bioactive compounds have garnered considerable interest in the food industry. Research is ongoing into their ability to alleviate problems such as oxidation, dehydration, rancidity, and browning. Consequently, these films not only improve the physicochemical, nutritional, and sensory properties of food products but also prolong their shelf life [66].

The light transmission results reveal that integrating NE-OELO into the CMC matrix has converted the material into a UV barrier film. This characteristic is highly sought after in the active packaging market. This film is especially effective in preserving photosensitive foods such as dairy products, processed meats, oils, and spices, as it effectively blocks nearly 100% of UV radiation, particularly at a wavelength of 280 nm.

Nevertheless, the decrease in transparency in the visible spectrum may restrict its application in foods where clear visibility is crucial, even though the market often prioritizes quality protection over complete transparency for many perishable products.

In summary, the active film developed, particularly at higher concentrations such as F15 and F20, demonstrates optical properties that meet market demands for photoprotective and active packaging. This film offers significantly enhanced value in comparison to unmodified CMC film.

3.6 Thermogravimetric Analysis

Thermal stability is a crucial consideration in the industrial application of active films, as temperature fluctuations can occur during processing and storage. Thermogravimetric Analysis (TGA) enables the evaluation of a material's thermal stability and the effects of incorporating a nanoemulsion into the polymer matrix. Figure 3 illustrates the TGA and derivative thermogravimetric (DGT) curves for the components involved in the film formulation: (A) Carboxymethylcellulose (CMC), (B) Glycerol, and (C) *Lippia organoides* Essential Oil Nanoemulsion (NE-OELO).

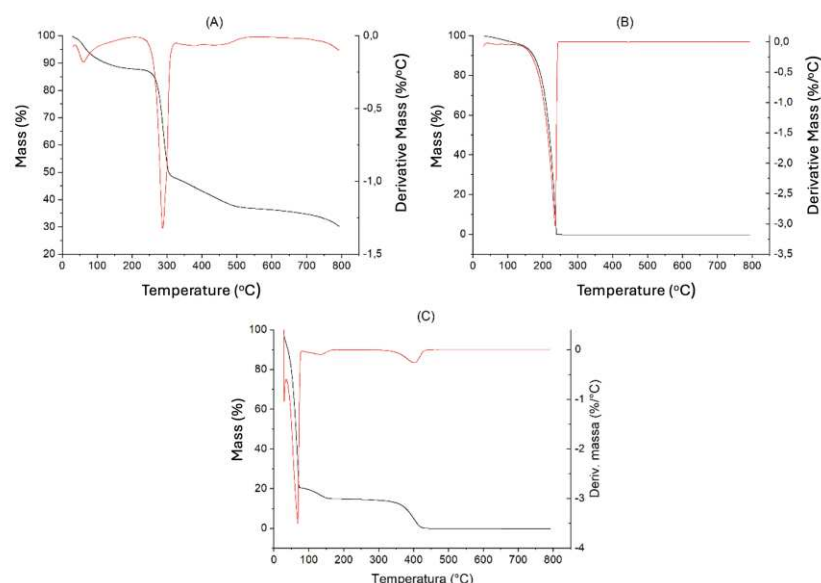


Fig. 3 TGA curves of the compounds used in the film development: (A) carboxymethylcellulose, (B) glycerol, and (C) a nanoemulsion of *Lippia organoides* essential oil.

Thermogravimetric analysis revealed three primary temperature ranges for each material. For carboxymethylcellulose (A), the initial mass loss occurs up to approximately 120 °C, which is linked to the release of physically adsorbed water. The main mass loss event occurs between approximately 220 °C and 350 °C, exhibiting a sharp peak near 300 °C. This peak is indicative of polymer chain cleavage and the decarboxylation of functional groups. Beyond 350 °C and up to

800 °C, mass loss is attributed to oxidation and the removal of remaining carbonaceous residues. This multi-stage decomposition is typical of hydrophilic polymers, such as carboxymethylcellulose, highlighting processes including residual water loss, oxidative degradation of the polymer chain, and the breakdown of side functional groups [67-69]. These observations demonstrate the material's structural integrity and enhanced resistance to thermal degradation, making it an attractive option for biodegradable packaging applications.

Glycerol (B) exhibits nearly complete mass loss at temperatures up to approximately 200 °C, resulting in rapid volatilization and thermal degradation, with the primary decomposition event occurring before reaching 250 °C. This behavior is characteristic of plasticizing agents, which are incorporated into polymeric compounds to alter the polymer matrix and enhance chain mobility for gelatinization [70]. Such modifications reduce the intermolecular attractive forces among polymer molecules, thereby diminishing the thermal resistance of films plasticized with glycerol [72].

In graph C, which illustrates the nanoemulsion (NE OELO), there is an initial loss of water and solvents up to approximately 120 °C. Between 120 °C and 270 °C, evaporation and transformation of the organic components in the *Lippia organoides* essential oil, along with the surfactant (Tween 80) used in the formulation, occur. It was observed that changes and oxidation of organic residues and lipid phases in the temperature range above 300 °C and up to 800 °C, which define the thermal profile of the system. The thermogravimetric analysis results suggest that the nanoemulsion remains thermally stable only at lower temperatures.

Recent studies underscore the thermal degradation pattern observed in essential oil nanoemulsions within polymer films. A developed nanoemulsion containing *Lippia organoides* essential oil demonstrated stability only at lower temperatures. At extreme temperatures, it experiences mass loss due to the decomposition of organic components, resulting in the absence of significant residue [41].

While nanoemulsions exhibit limited thermal stability, utilizing nanostructured systems of essential oils within polymers to create films can effectively safeguard the active components [14]. These systems facilitate the

controlled release of volatile compounds, resulting in no residual mass after exposure to high temperatures, unlike films produced directly with essential oils.

Volatilization of essential oils occurs at relatively low temperatures, indicating that the incorporation of NE-OELO should take place under mild conditions. Furthermore, any sealing or packaging processes that involve heat must be controlled and maintained at a temperature below 100°C. This is crucial for minimizing the loss of carvacrol and other bioactive compounds, thereby ensuring the product's integrity and quality. Consequently, this film is particularly suitable for applications in foods that are not thermally processed after packaging, such as fresh or refrigerated items, where the release of bioactive compounds at room temperature or lower is optimized.

The study highlights the importance of nanotechnology in stabilizing the *Lippia origanoides* essential oil. However, the volatility inherent in monoterpenes remains an operational limitation to be considered on an industrial scale.

Analysis of the thermograms of active films incorporating different concentrations of NE-OELO (A-F0%, B-F10%(w/w), C-F15%(w/w), and D-F20%(w/w)) shows the direct influence of NE-OELO on the physicochemical properties and thermal stability of the developed films (Figure 4).

All films experience three primary stages of degradation. The loss of adsorbed water characterizes the initial stage of the process. The intermediate stage involves the decomposition of the functional groups of carboxymethyl cellulose (CMC) alongside the organic components of NE-OELO. Lastly, the final stage encompasses the degradation of the residual polymeric matrix.

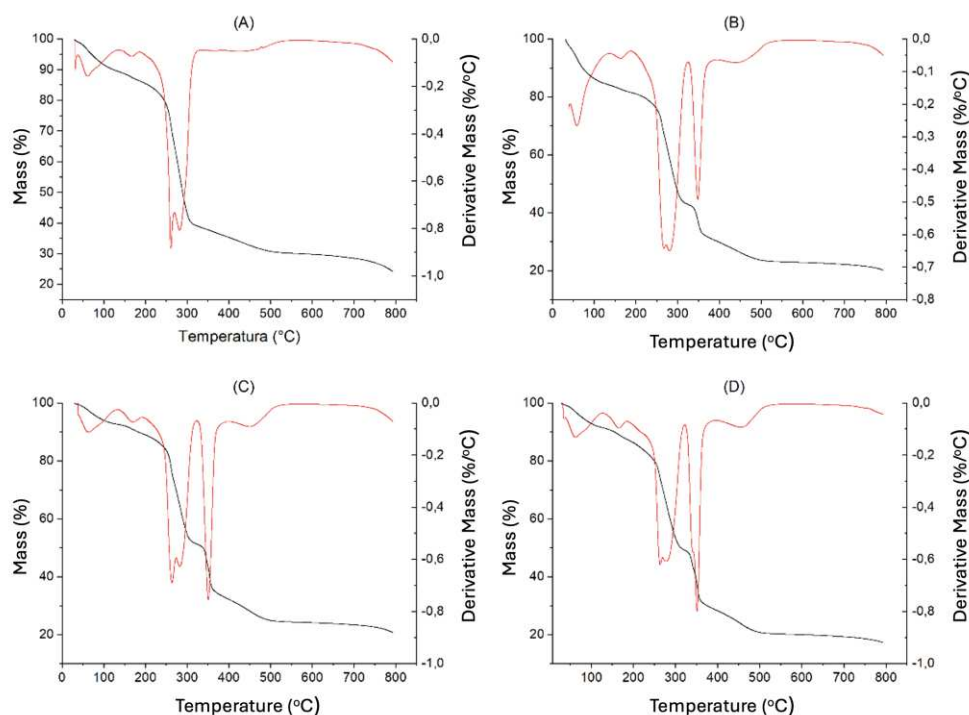


Fig. 4 TGA curves of carboxymethylcellulose films infused with varying concentrations of *Lippia origanoides* essential oil nanoemulsion. The samples are designated as follows: (A) control film (0% NE-OELO), (B) Film 10 (10% NE-OELO w/w), (C) Film 15 (15% NE-OELO w/w), and (D) Film 20 (20% NE-OELO w/w).

Thermogravimetric analysis of carboxymethylcellulose (CMC) films containing varying concentrations of NE-OELO highlights subtle yet noteworthy differences among the formulations.

The control film (graph A) displays three distinct stages of decomposition. The initial mass loss occurs up to approximately 120 °C, which is attributed to the removal of water that is physically adsorbed within the polymer matrix. A peak is observed between approximately 220 °C and 350 °C, suggesting the degradation of the polymer through the decarboxylation of its functional groups. Above 350 °C, a prolonged phase of residual carbon degradation occurs, characterized by a gradual rate of mass loss extending up to 800 °C.

In Graph B, the film containing 10% NE-OELO exhibits an initial water elimination range similar to that of the control sample, reaching up to 120 °C. In the temperature range between 220 °C and 350 °C, multiple peaks are observed. This behavior suggests that, in addition to the manipulation of the CMC matrix,

there is an overlap in the suspension of volatile and phenolic constituents of the essential oil, which is reflected in the multiple thermal events seen in the curve. The final oxidation phase of residues above 350 °C shows a decrease in residual mass, indicating the presence of more thermolabile fractions.

The F15 film (graph C) demonstrates a profile that closely resembles that of the F10 film, but with even greater peak intensities in the 220–350 °C range. This observation reinforces the role of OELO components in the release of volatile compounds. Notably, the mass loss appears to be more substantial, and the proximity of the peaks at similar temperatures suggests a more substantial influence from the lipid phase. In the final stage, beyond 350 °C, the residues exhibit continuous oxidation behavior, with the F15 film showing an even lower residual mass than that of the F10 film.

In the F20 film illustrated in graph (D), the initial mass loss occurs up to 120 °C, corresponding to the removal of free water and moisture adsorbed within the polymeric matrix. Between 220 °C and 350 °C, multiple peaks are detected, probably reflecting the degradation processes associated with the depolymerization of carboxymethyl cellulose (CMC) and the breakdown of thermolabile constituents in OELO, including terpenes and phenolic compounds. The addition of 20% NE-OELO results in a greater release of volatile organic compounds and a reduction in thermal stability. Above 350 °C, there is continuous degradation of residual carbon and lipid fractions.

Films containing nanoemulsion display multiple degradation peaks during the TGA test. This variability is attributed to the presence of encapsulated organic components. In contrast, the control film exhibits a more uniform decomposition pattern [72].

This shift in the thermal profile indicates that the incorporation of the nanoemulsion alters the degradation dynamics of the film. This is attributed to the presence of volatile compounds and structural changes that promote the breaking of polymeric bonds at intermediate temperatures [73].

The results of this research reveal that, although the incorporation of NE-OELO enhances the functional properties of the active film—particularly its antioxidant and antimicrobial characteristics—there is a progressive decline in thermal stability as the concentration of organic components rises. This

observation underscores the need for future formulations that address this challenge, thereby facilitating potential industrial applications.

The thermal properties obtained are well-suited to the niche market of packaging for refrigerated or fresh foods that are not subjected to sterilization or heating after filling. In this segment, the key factors are the physical stability of the film (its resistance) and the effective release of the active ingredient under low temperature conditions. This work is noteworthy for characterizing a system that maintains physical stability for the active component while being thermally vulnerable, thus guiding its application in a technically and scientifically sound manner.

3.7 X-ray Diffraction (XRD)

The crystallinity index (CI) of the films averaged 12.2 ± 1.6 , suggesting that the carboxymethylcellulose (CMC) films integrated with *Lippia origanoides* essential oil nanoemulsion possess a predominantly amorphous structure, characterized by only a few detectable crystalline domains (Figure 5). Notably, the addition of the nanoemulsion did not result in significant alterations to the crystalline organization of the polymeric matrix.

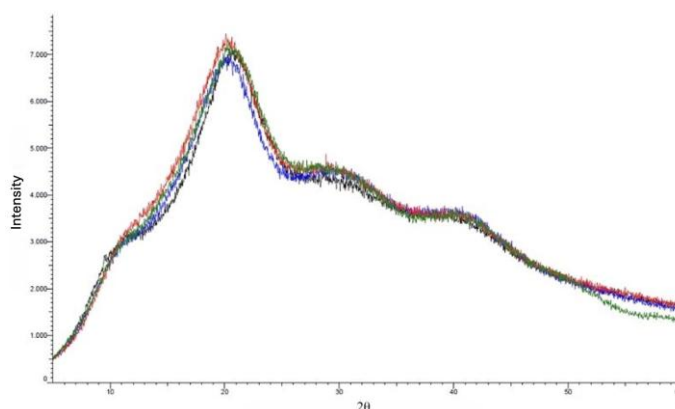


Fig. 5 X-ray diffractograms (XRD) of carboxymethylcellulose films infused with various concentrations of *Lippia origanoides* essential oil nanoemulsion. The control film: 0% NE-OELO (black); films with different concentrations include 10% NE-OELO (blue), 15% NE-OELO (red), and 20% NE-OELO (green). This figure illustrates how the incorporation of the essential oil affects the structural organization of the polymeric matrix.

Comparable results were described in literature. Carboxymethylcellulose-based composite films that included a nanoemulsion of *Cinnamomum* (cinnamon) essential oil. Their XRD tests revealed that the control film displayed

a broad diffraction peak at 20°, indicating a crystallinity of approximately 17% and suggesting a low crystalline structure of carboxymethylcellulose (CMC). Importantly, the introduction of *Cinnamon* essential oil did not lead to the emergence of any new diffraction peaks [46]. Moreover, XRD analysis of carboxymethylcellulose (CMC) nanocomposite films confirmed the amorphous nature of CMC, noting that the intensity of the characteristic peak of the control polymer did not change upon the incorporation of active compounds [74].

The results of this study suggest that the films exhibit a disordered, flexible, and transparent structure. In this configuration, the dispersion of oil components (terpenes and phenols) physically interacts with the polymer chains, preventing crystalline packing while preserving the structural topology of the matrix [48].

3.8 Antioxidant Activity

Evaluating the antioxidant capacity of packaging films is crucial, as lipid oxidation is a primary cause of food spoilage and a contributor to reduced shelf life. Table 4 illustrates the antioxidant capacity of carboxymethylcellulose (CMC) films incorporating various concentrations of *Lippia origanoides* essential oil nanoemulsion (NE-OELO). This assessment was carried out using the DPPH and ABTS radical scavenging assays.

Table 4 – Antioxidant capacity of carboxymethylcellulose films incorporating different concentrations of *Lippia origanoides* essential oil nanoemulsion.

Film	Antioxidant capacity (μmol TE/g _{filme})	
	DPPH	ABTS
F0	nd	1,71 ± 0,07 c
F10	10,54 ± 0,73 c	189,32 ± 44,51 b
F15	23,08 ± 1,88 b	236,03 ± 41,97 b
F20	35,89 ± 0,62 a	389,55 ± 37,58 a

*In the columns, means that share the same letters do not differ statistically from each other, according to Tukey's test at the 5% probability level. DPPH refers to 2,2-diphenyl-1-picrylhydrazyl radical; ABTS refers to 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) cation. **nd: not detected. F0: control film (0% NE-OELO), F10 (10% NE-OELO w/w), F15 (15% NE-OELO w/w), and F20 (20% NE-OELO w/w).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is widely utilized to evaluate a compound's ability to donate hydrogen or electrons. In this study, the pure carboxymethyl cellulose (CMC) film, designated as F0, exhibited no capacity to scavenge DPPH radicals, indicating that the base polymeric matrix possesses minimal intrinsic antioxidant activity.

In contrast, the incorporation of NE-OELO resulted in a detectable antioxidant activity across all tested concentrations. A dose-dependent relationship was observed, with antioxidant capacity increasing in proportion to the concentration of NE-OELO. Among the films examined, Film F20 exhibited the highest DPPH scavenging activity, with a value of $35.89 \pm 0.62 \mu\text{mol TE/g}$ film, significantly outperforming both F15 and F10.

To validate the findings, an assay was performed using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) cation radicals (ABTS), which provides a thorough evaluation of antioxidant potential across both lipophilic and hydrophilic phases. The results revealed that the antioxidant capacity increased with the addition of NE-OELO, confirming the efficacy of the nanoemulsion as a carrier for bioactive compounds.

The enhanced antioxidant activity observed in the ABTS assay can be attributed to its superior sensitivity, as it evaluates both hydrophilic and lipophilic compounds, unlike the DPPH assay, which primarily measures responses in the organic phase. Consequently, the detection of antioxidant activity in the control film exclusively in the ABTS assay may be due to the presence of hydroxyl groups and reducing residues of CMC, which react with the ABTS^+ radical but not with DPPH^- [75,76].

The results indicate that the incorporation of NE-OELO has transformed the CMC film from an inert material into an active and functional film with high antioxidant capacity. The packaging market increasingly demands solutions that can extend the shelf life of products. The active film's high antioxidant capacity enables it to serve as a "free radical scavenger" at the interface between food and packaging, thereby preventing or delaying lipid oxidation and rancidity—issues that are particularly common in high-fat foods.

Our research group has previously identified a total of twenty distinct compounds in the essential oil derived from *Lippia organoides*. The predominant constituents include carvacrol at 41.56%, thymol at 12.56%, and p-cymene at

12.49%. Additionally, significant components such as β -caryophyllene and γ -terpinene constitute 6.61% and 6.27%, respectively.

This study indicates that the effectiveness of NE-OELO is, in part, attributed to its primary component, carvacrol. A recent literature review [77] noted that carvacrol, a phenolic monoterpene abundant in essential oils like that of *Lippia origanoides*, is recognized for its versatility and potent antioxidant properties. Moreover, the review highlights carvacrol's promising role as an additive in the food industry, where it effectively scavenges reactive oxygen and nitrogen species, thereby protecting foods from lipid oxidation and undesirable changes in taste and aroma.

The authors emphasize that, despite carvacrol's considerable potential, its direct incorporation into food products or packaging is frequently hindered by several inherent challenges. These challenges include its high volatility, low water solubility, and a pronounced aroma and flavor that may be undesirable. To address these limitations, a nanoencapsulation strategy utilizing nanocarriers, such as nanoemulsions, presents a technological solution.

Hence, the findings of this study underscore the effectiveness of CMC-based active films incorporating NE-OELO at various concentrations for food packaging applications, particularly for items that are sensitive to oxidation.

3.9 Antimicrobial Activity

The antimicrobial activity of the volatile compounds found in active films containing NE-OELO was tested against *Staphylococcus aureus* (ATCC 00039), *Escherichia coli* ETEC (ATCC 11105), and *Salmonella typhimurium* (ATCC 14028), as described in the methodology.

Figure 6 illustrates that the number and distribution of *S. aureus* colonies steadily decreased as the concentration of the nanoemulsion in the active film increased. In contrast, the control film (without NE-OELO) exhibited uniform colony growth, indicating a lack of antimicrobial activity. Additionally, no growth inhibition was observed for the Gram-negative bacteria *Escherichia coli* ETEC (ATCC 11105) and *Salmonella typhimurium* (ATCC 14028).

Natural antibacterial agents, such as essential oils, can interact with the hydrophobic core of lipid layers, thereby altering the physicochemical properties of cellular membranes. This interaction can result in membrane ruptures, leakage

of cellular contents, deformation, and cellular swelling, which suggests a process of membrane permeabilization [78-80]. Additionally, essential oils penetrate the permeable peptidoglycan layer of Gram-positive bacteria more readily than they do the complex outer membrane of Gram-negative bacteria [81,82].

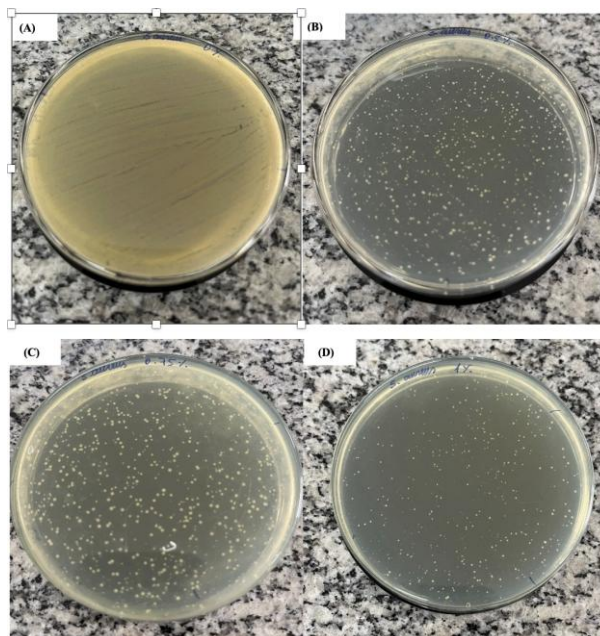


Fig. 6 Volatilization assay of carboxymethylcellulose films that have been incorporated with different concentrations of *Lippia origanoides* essential oil nanoemulsion. The films include: (A) the 0% control film (0% NE-OELO), (B) Film 10 containing 10% NE-OELO (w/w), (C) Film 15 containing 15% NE-OELO (w/w), and (D) Film 20 containing 20% NE-OELO (w/w) against *Staphylococcus aureus*.

Moreover, the antimicrobial activity observed is often attributed to compounds such as carvacrol and thymol, which are present in the chemical composition of NE-OELO. Biodegradable films enriched with carvacrol and thymol were evaluated for their antimicrobial properties, demonstrating significant inhibition of food spoilage bacteria, including *Staphylococcus aureus* [13].

A film composed of cellulose, carboxymethyl cellulose (CMC), and thymol, exhibited an antimicrobial inhibition zone of 17.1 mm against the *Staphylococcus aureus* strain [83]. This finding underscores the enhanced antimicrobial properties achieved by incorporating thymol into the formulation of active films.

Although there is currently no reported antimicrobial activity against Gram-negative bacteria, it is essential to emphasize that *Staphylococcus aureus* is a Gram-positive pathogen of significant clinical importance. It is also one of the leading causes of food poisoning on a global scale [84].

The primary concern centers on strains of *Staphylococcus aureus* that produce staphylococcal enterotoxins (SEs), which are heat-stable proteins. Even after cooking, SEs that are pre-formed in food can remain active and lead to illness. Staphylococcal food poisoning is characterized by a rapid onset of nausea, vomiting, and abdominal cramps, typically occurring 30 minutes to 8 hours after consuming contaminated food [85].

Contamination by *S. aureus* is often associated with shortcomings in good hygiene and handling practices, as humans are significant asymptomatic carriers of the bacteria, which can reside in their nasal cavities, throats, skin, and hair. Foods that involve considerable handling, such as sliced meats, dairy products, creams, salads, and sandwiches, are frequently implicated, particularly when stored at inadequate temperatures that facilitate bacterial growth and toxin production [86]. This highlights the importance of implementing effective control strategies, such as active packaging, to minimize the risk of contamination.

The findings suggest that natural resources, particularly nanoemulsified essential oils, hold substantial biotechnological promise for the development of active packaging solutions that enhance food preservation and ensure consumer safety.

It is crucial to underscore that the diverse and complex chemical compositions of these oils play a significant role in reducing the risk of microbial resistance, making it challenging for microorganisms to adapt [87]. Consequently, the findings of this research hold particular importance for the development of natural alternatives that combat antibiotic-resistant pathogens affecting the food industry.

3.10 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was conducted on the active films to examine potential changes in molecular vibrational modes resulting from the incorporation of varying concentrations of NE-OELO into the carboxymethylcellulose (CMC) polymer matrix. This spectroscopic analysis is necessary for confirming the effective integration of the active ingredient and for identifying intermolecular interactions that affect the stability and functional performance of the active film (Figure 7).

The spectrum of the control film (F0) exhibited the characteristic bands of CMC, with a prominent broad absorption peak around 3400 cm^{-1} , attributable to O–H vibrational stretching. Additionally, bands observed near 1600 and 1410 cm^{-1} correspond to the asymmetric and symmetric vibrations of the carboxylate group (COO^-), thereby confirming the structural integrity of the base polymer.

The incorporation of NE-OELO at varying concentrations (10%, 15%, and 20% w/w) led to noticeable changes in the corresponding spectral regions, observed as a broadening of the O–H band ($3600\text{--}3000\text{ cm}^{-1}$) and an increase in intensity in the range of $1200\text{--}1000\text{ cm}^{-1}$. These observations indicate an enhancement in hydrogen bonding and improved miscibility between the polymeric and lipid phases. Such behavior is typical of the presence of natural hydroxylated agents [88,89].

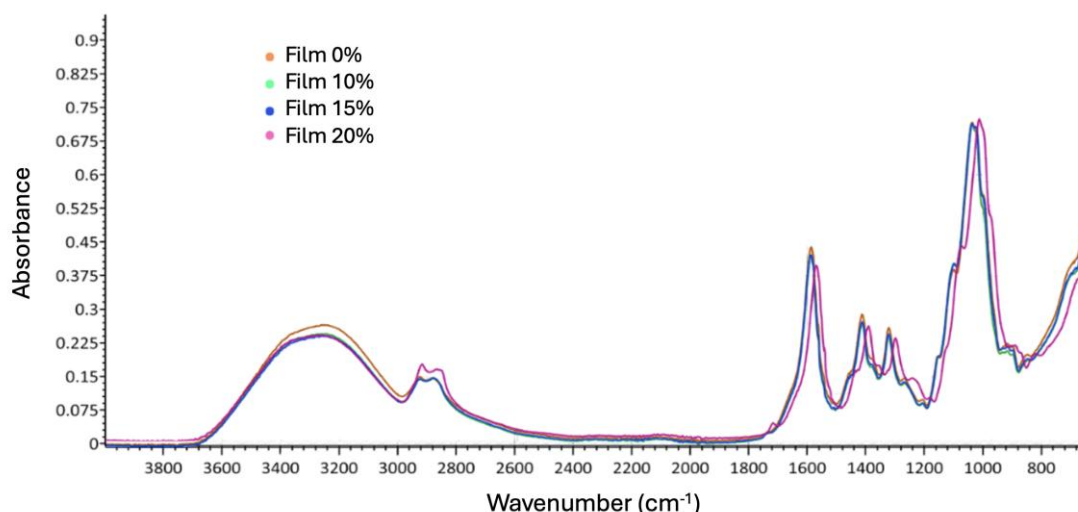


Fig. 7 FT-IR spectra of carboxymethylcellulose films that incorporate different concentrations of polymeric nanoemulsion containing *Lippia organoides* essential oil. The different films are as follows: the control film (0% NE-OELO), Film 10 (10% NE-OELO w/w), Film 15 (15% NE-OELO w/w), and Film 20 (20% NE-OELO w/w).

No FT-IR analysis results for active films containing NE-OELO were referenced in the literature at the time of writing this article for comparative purposes. A research about a biopolymeric film that incorporates thymol and carvacrol, the major compounds found in *Lippia organoides* essential oil, reported that both thymol and carvacrol interact with the polymeric matrix, forming

intermolecular hydrogen bonds between their terminal hydroxyl groups and the carbonyl groups of the polymeric esters. The incorporation of these essential oils led to the emergence of new peaks, an increase in intensity, and shifts in the primary bands [13].

The FT-IR spectra of the various films containing NE-OELO exhibited a broad O–H band within the range of 3600 to 3000 cm^{-1} , indicating the presence of a substantial network of hydrogen bonds. Additionally, characteristic carboxylate pairs were observed at approximately 1600 and 1410 cm^{-1} . The incorporation of NE-OELO at concentrations of 10–20% resulted in an even greater broadening of the O–H band and an increase in the intensity of the envelope between 1200 and 1000 cm^{-1} . The heightened intensity in the regions near 1600 and 1510 cm^{-1} can be attributed to the vibrational activity of the aromatic ring of carvacrol, the major component of OELO. Furthermore, a peak around 1260–1230 cm^{-1} corresponding to the phenolic $\nu\text{C–O}$ was observed, confirming the effective incorporation of the *Lippia origanoides* essential oil nanoemulsion.

The results are consistent with literature [13], which described that the incorporation of essential oil induces changes in both asymmetric and symmetric elongation, as well as in bending vibrations. This effect is likely attributed to the rearrangement of polymer chains that chemically interact with carvacrol.

FT-IR analysis plays a pivotal role in supporting that nanotechnology facilitates the uniform dispersion and molecular interaction of the essential oil within the hydrophilic CMC matrix. The strong evidence of hydrogen bonding interactions, along with the detection of carvacrol vibrational modes within the film, confirms that the functionalization of the matrix has been successfully achieved at the molecular level. It is suggested that this integration serves as the foundation for the material's superior biological performance, which directly influences its functional properties, such as controlled release of the active ingredient compared to the rapid volatilization associated with its pure form, and enhancements in antioxidant capacity and selective antimicrobial activity against *Staphylococcus aureus*, due to the molecular distribution and interactions of NE-OELO.

In summary, spectroscopy provides compelling evidence of molecular compatibility and miscibility, affirming the success of the nanotechnology approach in developing high-performance active films.

4. CONCLUSION

This work presents the formulation of a high-quality nanometric system for the effective incorporation of a lipophilic compound, specifically, an essential oil rich in carvacrol, into a hydrophilic polymeric matrix composed of carboxymethylcellulose (CMC).

The development and characterization of active films based on carboxymethylcellulose integrated with NE-OELO yielded functional materials with significant potential for food packaging applications. The nanoemulsions exhibited satisfactory homogeneity and stability, enhancing the physical, optical, and functional properties of the films without compromising key attributes such as transparency and flexibility.

Active films displayed effective barrier properties against ultraviolet radiation and successfully maintained transparency levels that comply with visual inspection standards. This feature is crucial for ensuring consumer acceptance and safeguarding food during storage. Evaluations of antioxidant activity revealed a direct correlation between the concentration of NE-OELO and the enhancement of antioxidant potential, which is attributed to the presence of phenolic compounds such as carvacrol. Moreover, antimicrobial activity assessments indicated that the inclusion of the nanoemulsion enabled the films to effectively inhibit the growth of the food-spoiling microorganism *Staphylococcus aureus*, demonstrating a concentration-effect relationship.

Thermal analyses revealed that the inclusion of the nanoemulsion alters the degradation profile of the films, underscoring the importance of evaluating their stability under practical application conditions. Although incorporating NE-OELO results in a reduction of mechanical strength, the materials retain sufficient flexibility for specific applications within food systems.

Among the formulations evaluated, F15 and F20 showed the best balance of physical and functional properties, with F15 standing out for its smaller impact on mechanical and optical properties.

This research contributes to a deeper understanding of nanostructured systems based on biopolymers and essential oils, demonstrating that the integration of nanoemulsions can serve as a promising strategy to enhance the functional properties of active packaging. Further studies should be conducted in food systems under real-world usage conditions to better assess the performance of the active film throughout its commercialization and consumption.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CLINICAL TRIAL NUMBER

Clinical trial number: not applicable.

This study did not involve a clinical trial, human intervention, or animal experimentation. The research was conducted exclusively through laboratory analyses for the development and characterization of carboxymethylcellulose films incorporated with *Lippia organoides* nanoemulsion for application as active food packaging materials.

CONSENT FOR PUBLICATION

Consent for publication: not applicable.

This manuscript does not contain individual-level data from human participants, identifiable images, or personal information. Therefore, consent for publication was not required.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The collection of *Lippia origanoides* plant material followed Brazilian regulations and guidelines on the collection and use of plant genetic resources. We sourced this material from a wild population in the municipality of Riachão do Jacuípe, Bahia, Brazil, in May 2024. Access to the Brazilian genetic heritage was duly registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the registration code A4F716D. The taxonomist Lamarck do Nascimento Galdino da Rocha formally identified the species. The authenticated specimen is deposited in the Herbarium Collection of the Department of Biology at the Universidade Estadual de Feira de Santana, with voucher accession number HUEFS259034. We followed all applicable requirements for collecting and accessing the plant material.

GENETIC HERITAGE ACCESS

The collection and use of *Lippia origanoides* complied with the applicable Brazilian regulations governing access to genetic heritage. The access to the Brazilian genetic heritage was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration code A4F716D.