

Effect of eugenol acetylation on encapsulation, controlled release, and antimicrobial properties of alginate films incorporating lignin microparticles

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Abstract

In this work, lignin microparticles were synthesized using the antisolvent method for the encapsulation of eugenol and acetyl eugenol. Acetyl eugenol was synthesized by esterification and characterized by spectroscopic techniques (^1H NMR, ^{13}C NMR, and two-dimensional NMR). Lignin microparticles exhibited spherical morphology with an average size of approximately 2 μm . The obtained microparticles were subsequently incorporated into sodium alginate films aimed at developing materials with antimicrobial activity. The encapsulation efficiency (EE) increased with the initial drug loading, and the higher EE of acetyl eugenol was attributed to its lower polarity and higher affinity for the hydrophobic domains of lignin. *In vitro* drug release studies revealed different profiles: acetyl eugenol exhibited a faster release rate, whereas eugenol presented a slower release because hydrogen-bond interactions with lignin delayed diffusion. Alginate films exhibited a homogeneous surface and excellent thermal stability, and the incorporation of lignin microparticles into the film was confirmed by FTIR and microscopy studies. Microbiological assays demonstrated that the composite materials maintained the antimicrobial activity compared to the free active compounds, with pronounced bactericidal effect against *Staphylococcus aureus* ATCC 29213. These results highlight the potential of lignin microparticles as controlled-release platforms for the fabrication of active materials for food packaging applications.

HIGHLIGHTS

Acetyl eugenol was synthesized and microparticles based on ionic lignin were prepared using the antisolvent method.

Acetyl eugenol exhibited higher encapsulation efficiency than eugenol.

A controlled release profile was observed, with eugenol exhibiting slower diffusion, while acetyl eugenol exhibited faster release.

Incorporation of lignin microparticles into alginate films produced materials with excellent antimicrobial activity.

1. INTRODUCTION

Food waste represents a growing concern for global food security and the sustainability of production systems, with losses reaching 89 million tons annually in Europe due to spoilage and shelf-life expiration¹. These processes are governed by intrinsic factors (such as water activity, pH, redox state, and nutrient content) and extrinsic factors (including temperature, humidity, and gaseous composition), which promote oxidation and microbial growth, ultimately deteriorating the sensory and nutritional quality of foods. Conventional packaging, largely derived from non-biodegradable polymers, also poses significant environmental challenges, driving the development of sustainable materials capable of acting as functional barriers^{2–4}.

In this context, active packaging systems are defined by European regulations as materials that incorporate substances able to release or absorb compounds to extend food shelf life, and have gained increasing attention for food packaging applications, particularly through the incorporation of natural antioxidants and antimicrobials ⁵. Essential oils and their related components stand out as safe and effective natural compounds for active packaging formulation ⁶. Among them, clove essential oil (rich in eugenol) exhibits well-established antioxidant and antimicrobial properties ^{7–10}. Eugenol is approved for use in food-contact materials (EU Regulation 10/2011), and has anti-inflammatory, antifungal, antiviral, antibacterial, and antioxidant activities, associated with its ability to scavenge free radicals and inhibit lipid peroxidation processes ^{11–14}. However, its high volatility, sensitivity to light and heat, and strong aroma limit its application in the formulation of active packaging systems ^{15–17}.

These drawbacks have motivated encapsulation strategies aimed at protecting the bioactive compounds and controlling their release profiles, particularly considering that the hydrophobic nature of essential oils often requires high dosages to achieve antimicrobial efficacy without compromising sensory attributes ^{18–20}. Moreover, although clove oil and eugenol exhibit remarkable activity against various bacterial strains, several studies have demonstrated that their efficacy can be significantly enhanced when they are used in combination with other antibacterial agents, indicating synergistic interactions ^{21–23}. In this regard, structural modification of eugenol through acetylation represents an attractive approach compared to pristine eugenol. Acetyl eugenol, rendered less polar by esterification of the hydroxyl group, may exhibit different stability, affinity towards polymeric matrices, and release behavior. In this regard, the comparative performance of these active compounds in drug delivery systems remains unexplored.

Biodegradable and renewable materials have attracted increasing interest as carriers for the controlled release of active principles. In recent years, the valorization of lignin, one of the major components of plant biomass, has been investigated which are relevant for several applications. Its abundance, low cost, renewable nature, and aromatic structure rich in phenolic functional groups confer intrinsic antioxidant properties and the ability to establish non-covalent interactions, such as hydrogen bonding and π – π stacking, relevant for the retention and stabilization of bioactive compounds ²⁴. Previous studies have shown that lignin incorporation into polymer matrices improves their mechanical performance, water vapor barrier properties, and UV protection ^{25–28}, while its conversion into micro- or nanoscale particles enhances surface area, compatibility with biopolymers, and antioxidant functionality ^{29,30}. In particular, microparticles produced by antisolvent methods offer advantages in terms of simplicity, reproducibility, and scalability ^{31,32}. Despite the growing interest in lignin-based carriers, the use of ionic lignin microparticles for the encapsulation of eugenol derivatives has not been systematically investigated. It remains unclear how structural modifications of phenolic molecules influence their interaction with lignin matrices, and how these interactions govern encapsulation efficiency, release kinetics and antimicrobial performance.

In this context, the present study aims to develop a lignin-based microencapsulation platform for eugenol and acetyl eugenol using an antisolvent precipitation approach. The resulting microparticles are incorporated into sodium alginate films to produce antimicrobial materials. The systems are systematically evaluated in terms of encapsulation efficiency, in vitro release profiles, and antimicrobial activity against *Escherichia coli* ATCC 8739 (*E. coli*) and *Staphylococcus aureus* ATCC 29213 (*S. aureus*). This study provides a direct comparison of eugenol and acetyl eugenol encapsulated in ionic lignin microparticles and incorporated into alginate films, establishing correlations between molecular structure, encapsulation efficiency, release behavior, and antimicrobial performance.

2. EXPERIMENTAL

2.1 Materials

The following reagents were used without further purification: eugenol (Sigma-Aldrich, Germany), glycerol (Cicarelli, Argentina), tetrahydrofuran (THF) (Cicarelli, Argentina), sodium dodecyl sulfate (SDS) (Cicarelli, Argentina), calcium chloride (CaCl_2) (Sigma-Aldrich, Germany), sodium alginate (Todo Drogas, Argentina), potassium bromide (KBr) (Cicarelli, Argentina), ethanol (Cicarelli, Argentina), pyridine (Sigma-Aldrich, Germany), dichloromethane (DCM) (Sintorgan, Argentina), 4-(dimethylamino)pyridine (DMAP) (Sigma-Aldrich, Germany), acetic anhydride (Cicarelli, Argentina), potassium bisulfate (KHSO_4) (Sigma-Aldrich, Germany), anhydrous sodium sulfate (Na_2SO_4) (Cicarelli, Argentina), ethyl acetate (Cicarelli, Argentina), hexane (Cicarelli, Argentina), and deuterated chloroform (CDCl_3) (Cicarelli, Argentina). Ultrapure water ($0.055\ \mu\text{S}/\text{cm}$) obtained from an OSMOION purification system was used for all dilutions.

Lignin was derived from softwood spruce and isolated through an ionic process. This biopolymer was characterized as previously reported by Taverna et al. (2018, 2024)^{33,34} using gravimetric, chromatographic (SEC), spectroscopic (FTIR, UV–Vis, and NMR), and thermogravimetric (TGA) techniques to determine moisture and ash content, molecular weight distribution, functional groups, and thermal properties, respectively. For microbiological assays, two representative bacterial strains were used: the Gram-negative strain *Escherichia coli* ATCC 8739 (*E. coli*) and the Gram-positive strain *Staphylococcus aureus* ATCC 29213 (*S. aureus*).

2.2 Synthesis and characterization of acetyl eugenol

Initially, a solution was prepared by stirring 10 g of eugenol in 2 mL of pyridine. Subsequently, 20 mL of DCM and 10 mg of DMAP were added, and the mixture was maintained under continuous stirring for 30 min at room temperature. Acetic anhydride was then introduced in a 1:1 molar ratio relative to eugenol, and the reaction was allowed to proceed under stirring for an additional 60 min. Upon completion, the excess pyridine was neutralized by the addition of a 10% KHSO_4 solution.

To remove water-soluble impurities, liquid–liquid extractions were performed using 20–30 mL portions of DCM. After each addition, the mixture was vigorously shaken and allowed to stand to enable phase

separation. The organic phase was retained for further processing, while the aqueous phase containing dissolved salts was discarded. The organic layer was subsequently washed with water until neutral pH was reached and dried over Na₂SO₄ to remove residual moisture. The solvent was then removed under reduced pressure using a rotary evaporator. Final purification was achieved by silica gel column chromatography using an ethyl acetate/hexane solvent gradient, yielding acetyl eugenol with a 60%.

Acetyl eugenol was characterized by proton nuclear magnetic resonance (¹H NMR), carbon-13 nuclear magnetic resonance (¹³C NMR), to elucidate the molecular structure of the isolated compound. Spectra were acquired using a Bruker UltraShield™ 400 NMR spectrometer (Bruker, Germany) operating at 300 MHz, with deuterated chloroform as the solvent.

2.3 Synthesis and characterization of lignin microparticles

Lignin particles were prepared using the antisolvent precipitation method. The methodology followed for microparticle fabrication is schematically illustrated in Fig. 1. Briefly, 0.2 g of lignin was dissolved in 10 mL of THF containing either 10 or 20 wt% of eugenol or acetyl eugenol as active compounds. A control sample without active principle was also prepared. In each case, the resulting solution was added dropwise to an aqueous SDS solution (0.1% w/v) at a flow rate of 1 mL·min⁻¹ using a peristaltic pump, under homogenization for 30 min at 3000 rpm using a high-shear homogenizer (POLYTRON PT 2500 E, Kinematica, Switzerland). The organic solvent was removed by vacuum evaporation using a rotary evaporator for 30 min at 25°C. The suspended microparticles were collected by centrifugation at 10,000 rpm for 5 min and washed with water to remove residual surfactant. Finally, the particles were dried for subsequent characterization.

The morphology and average size of the particles were evaluated by observing microparticle suspensions using an optical microscope (DM2500M, Leica, Germany) equipped with a digital camera (DFC290 HD, Leica). Approximately 200 microparticles per sample were measured to determine the mean particle size and size distribution using ImageJ image analysis software.

2.4 Encapsulation efficiency

The encapsulation efficiency (EE) was determined by dispersing approximately 3 mg of microparticles in 3 mL of ethanol and maintaining under stirring for 24 h to extract the active compound. The concentration of the active principle in the supernatant was quantified using the internal standard method in a gas chromatograph (5890 Series II, Hewlett-Packard) equipped with a capillary column (5% crosslinked PHME siloxane, 0.25 µm film thickness, 0.25 mm internal diameter, and 15 m length) and a flame ionization detector (FID), with nitrogen used as the carrier gas. The column temperature program consisted of an initial temperature of 70°C held for 1 min, followed by a ramp of 15°C·min⁻¹ to a final temperature of 280°C, which was maintained for 1 min. The EE was calculated as the percentage of encapsulated active compound relative to the initial amount added, according to Eq. (1):

$$EE = \frac{A_e}{A_i} \bullet 100$$

where A_e is the concentration of the encapsulated active compound and A_i is the initial concentration of the active compound.

2.5 Controlled release assays

The release profiles of eugenol and acetyl eugenol were investigated through *in vitro* release assays. Ultrapure water was used as the release medium, and the experiments were conducted at 25°C. The active-loaded lignin microparticles were dispersed in water, with the active compound corresponding to 20 wt% relative to lignin. Aliquots of 0.5 mL were withdrawn at predetermined time intervals from the initial time point. After each sampling, the extracted volume was immediately replaced with an equal volume of fresh water to maintain a constant total volume throughout the experiment. The concentration of the released compounds was quantified by gas chromatography using the same analytical method described for the EE determination.

2.6 Preparation and characterization of sodium alginate films

Figure 2 illustrates the schematic procedure for film preparation. Initially, a 2 wt% sodium alginate solution was prepared in distilled water at 60°C until complete dissolution. Glycerol was then added at 1 wt% as a plasticizing agent. Subsequently, lignin microparticles dispersed in 1 mL of water were incorporated into the solution, and the mixture was homogenized.

The composition of the films is presented in Table 1. The films were cast in silicone molds with a diameter of 6 cm and dried in an oven at 60°C for 24 h. Subsequently, the films were ionically crosslinked with CaCl₂ by immersion in 10 mL of a 2 wt% CaCl₂ solution. Finally, the crosslinked films were dried in an oven at 60°C for 24 h.

Table 1
Composition of films formulated with different lignin microparticles content.

Film 1	Sodium alginate (g)	Lignin microparticles (g)	Calcium chloride (g)
	0.10	-	0.20
Film 2	0.09	0.01	0.20
Film 3	0.08	0.02	0.20
Film 4	0.07	0.03	0.20

Film characterization included the following analyses: (i) morphological evaluation by optical microscopy and scanning electron microscopy (SEM); (ii) identification of characteristic functional groups by FTIR analysis; and (iii) thermal stability evaluation by TGA.

For SEM analysis, the samples were examined at an accelerating voltage of 10 kV and a working distance of approximately 4.5 mm using a Carl Zeiss Supra 35VP scanning electron microscope

(Germany).

FTIR spectra of the films were recorded in the range of 4000–500 cm^{-1} using the KBr pellet method with a Shimadzu FTIR-8201 PC spectrophotometer. Band assignments were performed according to Mansouri et al. (2006)³⁵.

Thermal stability tests were carried out using a thermogravimetric analyzer (TGA Q500 V20.13 Build 39) under an inert nitrogen atmosphere at a flow rate of 80 $\text{mL}\cdot\text{min}^{-1}$. The film samples were heated from 25 to 800°C at a heating rate of 10°C·min⁻¹.

2.7 Microbiological assays

The antimicrobial activity was evaluated by the agar disk diffusion method against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 29213 by measuring the diameter of the inhibition zones³⁶. Petri dishes containing tryptic soy agar medium were inoculated separately with each bacterial strain. The inoculum was prepared by direct colony suspension in saline solution to achieve a turbidity of 0.50 on the McFarland scale (equivalent to $1.5 \times 10^8 \text{ CFU}\cdot\text{mL}^{-1}$). The inoculum was evenly spread over the surface of solidified agar in Petri dishes using a sterile swab.

Three different experimental strategies were employed using 5 mm diameter samples: (i) filter paper discs impregnated with 5 μL of pure eugenol or acetyl eugenol; (ii) filter paper discs placed over 30 mg of loaded lignin microparticles previously deposited on agar; and (iii) sodium alginate films incorporating lignin microparticles loaded with the same active compounds. In all cases, control samples consisted of the corresponding formulations without active compound, while discs impregnated with pure eugenol or acetyl eugenol were considered as positive controls due to their higher concentration of active compound. The samples were aseptically placed onto the surface of the inoculated agar plates and incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of the inhibition zones using ImageJ software. For all situations exhibiting detectable inhibition halos, an additional viability assay was performed by aseptically collecting material from the inhibition zones and subculturing it on fresh tryptic soy agar plates, followed by incubation at 37°C for 24 h. The presence or absence of bacterial growth was then evaluated to differentiate between bactericidal and bacteriostatic effects.

3. RESULTS AND DISCUSSION

3.1 Lignin characterization

The ionic lignin exhibited a moisture content of 23.34%, an ash content of 0.6%, and an elemental composition of 66.26% C, 8.17% H, 0.26% N, and 0.71% S. Its macromolecular properties were characterized by a number-average molecular weight (M_n) of 768 $\text{g}\cdot\text{mol}^{-1}$, a weight-average molecular weight (M_w) of 3718 $\text{g}\cdot\text{mol}^{-1}$, and a dispersity of 4.84, indicating a broad molecular weight distribution³³.

FTIR analysis confirmed the presence of characteristic functional groups, with a broad intense band attributed to hydroxyl groups ($\sim 3400\text{ cm}^{-1}$), aliphatic C–H stretching vibrations in the $3000\text{--}3400\text{ cm}^{-1}$ region, and carbonyl-related signals between 1738 and 1709 cm^{-1} . In addition, aromatic skeletal vibrations were observed at $1605\text{--}1539$ and $1515\text{--}1505\text{ cm}^{-1}$, along with bands associated with syringyl and guaiacyl units in the $1340\text{--}1220\text{ cm}^{-1}$ range. Deformation bands of C–H groups and C–O stretching vibrations of primary and secondary alcohols were detected between 1130 and 1035 cm^{-1} . These phenolic and aromatic functionalities provide hydrogen-bonding and $\pi\text{--}\pi$ interaction sites for the potential retention of phenolic molecules.

Thermal behavior evaluated by TGA revealed an initial dehydration of approximately 1% between 20 and 100°C , followed by an additional mass loss of about 4% up to 220°C . The main degradation stage exhibited a maximum at 361°C , reflecting thermal stability typical of lignin-based materials with a high aromatic content ³⁴.

3.2 Characterization of acetyl eugenol

Figure 3 shows the ^1H NMR spectrum of acetyl eugenol, in which the chemical shifts (δ) corresponding to the hydrogen atoms are identified.

The band analysis of the ^1H NMR and ^{13}C NMR spectra was performed from the highest to the lowest chemical shift values.

^1H -NMR (300 MHz, CDCl_3 , δ ppm): 6.93 (d, $J = 7.8\text{ Hz}$, 1H, **(d)**-CH); 6.79 (m, 2H, **(g y e)**-CH); 5.95 (m, 1H, **(j)**-CH); 5.11 (m, 2H, **(k)**-CH); 3.82 (s, 3H, **(l)**- OCH_3); 3.37 (d, $J = 6.9\text{ Hz}$, 2H, **(i)**-CH); 2.31 (s, 3H, **(a)**- $\text{C}(\text{O})\text{CH}_3$).

^{13}C -NMR (75 MHz, CDCl_3 , δ ppm): 169.4 (**(b)**-C); 151.0 (**(h)**-C); 139.2 (**(j)**-C); 138.1 (**(c)**-C); 137.2 (**(f)**-CH); 122.7 (**(d)**-CH); 120.8 (**(e)**-CH); 116.3 (**(k)**-CH); 112.8 (**(g)**-CH); 55.9 (**(l)**- CH_3); 40.2 (**(i)**- CH_2); 20.8 (**(a)**- CH_3).

The analysis of the obtained results indicates that the synthesized compound corresponds to acetyl eugenol. The spectral bands are consistent with those reported in the literature ^{37,38}.

3.3 Characterization of lignin microparticles

3.3.1 Particle size and morphology

Figure 4 presents an optical micrograph of lignin microparticles. The particles exhibit a spherical morphology, with an average size of approximately $1.99 \pm 0.64\text{ }\mu\text{m}$. Moreover, it was confirmed that the incorporation of active compounds (eugenol or acetyl eugenol) did not produce significant changes in their mean particle size. Since particle size remained unchanged after eugenol or acetyl eugenol loading, differences observed in encapsulation and release can be attributed molecular interactions between lignin and the active compounds.

The literature indicates that the size of lignin particles depends mainly on the type of lignin used, the extraction method, and the synthesis procedure³⁰. It has also been reported that, in applications involving macroscopic matrices such as hydrogels³⁹, films²⁹, or Pickering-type emulsions⁴⁰, particles do not require nanoscale dimensions, since they are incorporated into larger systems where their primary function is related to active compound release or compatibilization. Stewart et al. (2014)³¹ demonstrated that both submicron particles and microparticles can be obtained by antisolvent precipitation, depending on variables such as SDS concentration, temperature, and sonication. However, the authors noted that when particle sizes approach 0.1–0.2 µm, additional safety considerations associated with food nanotechnology may arise, an area in which international guidelines remain under development according to the World Health Organization (2014). In this context, it is particularly relevant that the particles obtained in the present study exhibit an average size of approximately 2 µm, placing them clearly within the micrometer range and above the nanoscale threshold. Therefore, they do not fall within the scope of such safety concerns and are consistent with particle sizes traditionally employed in food-related applications.

3.3.2 Encapsulation efficiency

The results presented in Table 2 indicate that increasing the amount of incorporated eugenol or acetyl eugenol led to higher encapsulation efficiency. This behavior has been previously reported in lignin-based formulations and other biopolymer systems, where a higher proportion of hydrophobic compounds in the organic phase promotes their retention during particle precipitation or self-assembly, thereby reducing diffusion into the aqueous medium⁴¹. Acetyl eugenol showed the highest encapsulation efficiencies, reaching 23.06%. Acetylation decreases the polarity of the molecule, thereby increasing compatibility with the hydrophobic aromatic domains of lignin. Consequently, diffusion of the active compound toward the aqueous antisolvent phase is reduced during particle precipitation. In contrast, the systems containing eugenol displayed lower values, which may be attributed to its higher solubility in water and subsequent transfer to the aqueous phase during the antisolvent process. Similar results were reported by Simões et al. (2020)⁴² for eugenol-loaded microparticles, where the miscibility of the organic solvent with water caused significant losses of the active compound during emulsification, leading to reduced EE.

Table 2
Encapsulation efficiency of eugenol and acetyl eugenol in lignin microparticles.

Formulations	Active principle loading (% w/w)	EE (%)
1	Eugenol: 10	4.05 ± 0.20
2	Eugenol: 20	5.48 ± 0.54
3	Acetyl eugenol: 10	18.25 ± 2.50
4	Acetyl eugenol: 20	23.06 ± 2.47

3.3.3 Controlled release of the active principles

The *in vitro* release profiles of the active compounds are shown in Fig. 5. A gradual release over time was observed for both microparticle formulations. During the initial stage, both formulations exhibited a similar behavior, suggesting a rapid diffusion associated with the fraction located at or weakly bound to the particle surface. However, differences between the release profiles became evident after 200 min: acetyl eugenol reached 100% release at this time, whereas eugenol exhibited around 70% release, reaching approximately 90% after 400 min. Lignin contains phenolic, carbonyl, and carboxyl groups capable of establishing specific interactions with aromatic molecules such as eugenol, particularly through hydrogen bonding and π - π stacking interactions, which can enhance affinity and modify release kinetics. Moreover, the presence of hydroxyl groups in phenolic compounds has been reported to promote hydrogen bond formation with lignin or modified lignin materials, contributing to enhanced retention within the matrix ⁴³. In contrast, the release behavior of acetyl eugenol can be associated with the structural dynamics of lignin particles in aqueous environments. Several studies have demonstrated that lignin particles and colloids undergo hydration, swelling, and conformational rearrangements upon contact with water, which alter the internal microstructure and reduce the retention of molecules primarily bound through hydrophobic interactions. These processes facilitate the diffusion of compounds exhibiting lower affinity for lignin. In this context, acetyl eugenol, lacking a hydroxyl group and interacting through weaker associations, is more readily released once the matrix becomes hydrated and partially loses its structural rigidity ⁴⁴. Based on the controlled release studies, the release mechanism was further performed using regression analysis with different kinetic models. The best fit was obtained with the Higuchi model, with R^2 values of 0.9612 and 0.9745 for the eugenol- and acetyl eugenol-loaded formulations, respectively. This suggests that the encapsulated active compounds follow a diffusion-controlled release mechanism.

3.4 Characterization of sodium alginate film incorporating lignin microparticles

Figure 6 presents optical and SEM micrographs of sodium alginate films containing 10 and 30% lignin microparticles. The obtained films exhibited smooth surfaces and homogeneous particle dispersion throughout the polymer matrix. An increase in lignin content resulted in a more intense brown coloration of the films, consistent with the observations reported by Aadil et al. (2016) ⁴⁵ for alginate films containing different lignin proportions. Furthermore, the SEM micrographs suggest that the particles are embedded within the film structure, as no surface-deposited particles were observed.

The observed morphological homogeneity indicates high compatibilization between the polymer matrix and lignin microparticles, which favors the formation of continuous films without visible defects. Similar results were reported by Taverna et al. (2022) ⁴⁶, observing a uniform distribution of tar microparticles in sodium alginate films. Likewise, Lin et al. (2023) ⁴⁷ reported that the incorporation of sodium caseinate and trimethyl chitosan nanoparticles loaded with eugenol into gelatin matrices produced films with

smooth and compact surfaces, free of cracks and pores, indicating good compatibility among the components. In addition, Aadil et al. (2016)⁴⁵ described that the incorporation of lignin to alginate films yielded visually homogeneous materials without phase separation, exhibiting elastic properties and easy-to-handle, which was attributed to favorable interactions between alginate and lignin functional groups, enhancing the physicochemical and functional properties of the films.

Figure 7a shows the FTIR spectra of the different films. In all spectra, the characteristic absorption bands of sodium alginate can be observed at around 3200–3400 cm⁻¹ (O–H stretching), ~ 2900 cm⁻¹ (aliphatic C–H stretching), ~ 1590–1620 cm⁻¹ (asymmetric stretching of COO⁻), ~ 1400–1420 cm⁻¹ (symmetric stretching of COO⁻), and ~ 1020–1080 cm⁻¹ (C–O/C–O–C vibrations). These assignments are consistent with those reported in the literature for alginate-based films^{45,48,49}. After lignin incorporation, modifications in the spectra were observed due to the contribution of lignin characteristic bands, including the intensification of the broad band at 3200–3450 cm⁻¹ associated with phenolic O–H groups and the increase in aliphatic C–H stretching around ~ 2900 cm⁻¹. The band located near ~ 1700 cm⁻¹ is attributed to carbonyl stretching vibrations. In addition, the band corresponding to aromatic ring C=C stretching in the region of ~ 1500–1510 cm⁻¹, commonly used as a distinctive lignin marker, was clearly identified³⁴. For films containing 10, 20, and 30 wt% lignin particles, the intensity of this aromatic band increased progressively, confirming the successful incorporation of lignin into the film structure. In a similar manner, the integrated intensity of the broad O–H band at 3200–3450 cm⁻¹ increased with lignin content, consistent with the higher presence of phenolic hydroxyl groups. The broadening and slight shifts of this band suggest the occurrence of intermolecular hydrogen bonding interactions between alginate and lignin. Similar behavior has been previously reported, where lignin incorporation into alginate films led to modifications in the O–H region attributed to stronger intermolecular interactions within the polymer matrix⁴⁵. These interactions likely contribute to the homogeneous particle dispersion observed by microscopy and to the improved thermal stability discussed below.

The TGA curves of the prepared films are presented in Fig. 7b. Four main degradation stages can be identified in the thermograms. The first weight-loss step is observed approximately at temperature between 60–100°C and is associated with the evaporation of moisture present in the samples. The second degradation stage, observed around 190–250°C, is mainly attributed to the thermal decomposition of glycerol and sodium alginate, in agreement with previous works^{45,50–53}. This stage exhibits a maximum degradation rate at approximately 200°C, which shifts toward slightly higher temperatures with increasing lignin content, rising from 192°C for Film 1 to 200, 215, and 220°C for Films 2, 3, and 4, respectively, suggesting a stabilizing effect of lignin on the polymeric matrix. The third weight-loss event, occurring approximately between 300–600°C, corresponds primarily to the degradation of lignin. The complex and heterogeneous structure of lignin, including carbonyl, phenolic hydroxyl, and benzylic groups, caused a degradation profile over an extended temperature range⁵³. The fourth weight-loss stage occurs at high temperatures, in the range of 650–700°C. This stage is associated with the thermal decomposition of the material, leading to the formation of carbonaceous residues (char) and the formation of thermally stable inorganic structures resulting from ionic

crosslinking reactions ⁴⁵. Film 1 exhibits a total weight loss of approximately 85% at 700°C, leaving a carbonaceous residue of about 15%, whereas the char content increases with incorporation of lignin. This behavior indicates that the incorporation of lignin into alginate films contributes to an overall enhancement in thermal stability. The reported high thermal stability in films incorporated with lignin-based structures is associated with complex phenylpropanoid units, including aromatic phenyl and hydroxyl groups, which are very stable at high temperatures ^{53,54}. The improved thermal stability, together with the homogeneous particle dispersion observed by microscopy, indicates that lignin microparticles act not only as carriers for the active compounds but also as reinforcing components of the alginate matrix. These findings are consistent with the FTIR results, suggesting strong intermolecular interactions between alginate and lignin.

3.5 Antimicrobial properties of the composite films

Table 3 summarizes the inhibition halo diameters obtained for the three experimental strategies analyzed against *E. coli* and *S. aureus*.

Table 3		
Inhibition halo diameters* (mm) against <i>Escherichia coli</i> ATCC 8739 and <i>Staphylococcus aureus</i> ATCC 29213 for pure active compounds, encapsulated active principles in lignin microparticles, and sodium alginate films incorporating the lignin microparticles.		
Formulation	Inhibition halos [mm]	
	Gram-negative	Gram-positive
	<i>E coli</i> ATCC 8739	<i>S aureus</i> ATCC 29213
Filter paper + pure active compound		
<i>Eugenol</i>	19.87 ± 0.32	27.10 ± 0.21
<i>Acetyl eugenol</i>	7.79 ± 0.06	25.00 ± 0.10
Filter paper discs placed over lignin microparticles loaded with active compounds		
<i>Eugenol</i>	17.51 ± 2.04	16.10 ± 1.98
<i>Acetyl eugenol</i>	ND	7.54 ± 0.07
Sodium alginate films containing lignin microparticles loaded with the active principles		
<i>Eugenol</i>	15.77 ± 1.20	15.80 ± 2.26
<i>Acetyl eugenol</i>	ND	8.01 ± 2.03

*Inhibition halo diameter with bactericidal effect, including the 5 mm disk, expressed as the mean of 3 replicates ± standard deviation. Control samples without active compounds showed no inhibition halos. ND: No detectable inhibition halo.

As shown in Table 3, pure eugenol exhibited strong antimicrobial activity against both bacterial strains, producing inhibition zones of 19.87 ± 0.32 mm against *E. coli* and 27.10 ± 0.21 mm against *S. aureus*. In contrast, pure acetyl eugenol produced inhibition zones of 7.79 ± 0.06 mm against *E. coli* and 25.00 ± 0.10 mm against *S. aureus*. These results correspond to the positive controls, as the active compounds were applied at higher effective concentrations compared to the encapsulated formulations. This behavior is consistent with previous reports attributing the pronounced activity of eugenol against both Gram-negative and Gram-positive bacteria to its ability to disrupt membrane permeability and destabilize essential cellular components⁵⁵. Conversely, acetyl eugenol exhibited lower antimicrobial activity, particularly against *E. coli*, which may be associated with the absence of a free hydroxyl group, a functional moiety known to play a key role in the interaction of phenolic compounds with bacterial membranes^{55,56}.

Upon incorporation of the active compounds into lignin microparticles, differences in antimicrobial performance were observed. When applied using filter paper discs, eugenol loaded microparticles generated inhibition zones of 17.51 ± 2.04 mm against *E. coli* and 16.10 ± 1.98 mm against *S. aureus*, indicating a reduction in activity compared to the positive control. Encapsulation within lignin microparticles reduced the apparent antimicrobial activity compared with the corresponding free compounds. This decrease is expected because the active molecules must first diffuse from the lignin matrix before reaching the surrounding medium, resulting in a lower effective concentration during the early stages of the assay. In contrast, acetyl eugenol-loaded microparticles produced no detectable inhibition against *E. coli* and only a small inhibition zone against *S. aureus*. This behavior is consistent with the lower intrinsic antibacterial activity of acetyl eugenol, indicating that its higher encapsulation efficiency was insufficient to compensate for its reduced antibacterial activity.

The incorporation of lignin microparticles into the alginate films preserved the antimicrobial activity, indicating that diffusion of the active compounds through the hydrated polymeric network remained sufficient to inhibit bacterial growth. Although the inhibition zones were smaller than those produced by the free active compounds, they were comparable to those obtained using isolated lignin microparticles, suggesting that the alginate matrix did not introduce a substantial additional diffusion barrier. This behavior may be related to reduced volatilization of the encapsulated compounds and their gradual diffusion from the hydrated alginate matrix, resulting in lower initial effective concentrations but sustained release over time, which could contribute to maintaining antibacterial effectiveness even when smaller initial inhibition halos are observed.

Additionally, the reduced or non-detectable inhibition halos observed against *E. coli* in some formulations may be associated with the combination of limited diffusion of hydrophobic compounds within the agar medium and their lower effective concentration upon release, together with the intrinsic resistance of Gram-negative bacteria. Nevertheless, microencapsulation has been shown to enhance the stability and sustained antimicrobial performance of essential oil-derived compounds, improving their accessibility to bacterial targets over time despite lower initial inhibition zones^{57,58}.

Complementary to the inhibition zone analysis, the viability assay showed that no bacterial regrowth of *E. coli* or *S. aureus* was observed in subcultures obtained from the inhibition zones. The results indicate that the antimicrobial effect of the evaluated systems was bactericidal under the tested conditions.

4. CONCLUSIONS

Lignin-based microparticles were prepared as carriers for eugenol or acetyl eugenol, which were subsequently embedded within sodium alginate films to produce antimicrobial active materials. The particles exhibited uniform spherical morphology and micrometric size, and the encapsulation efficiency increased with the effective loading and the hydrophobicity of the active principle, reaching higher values for acetyl eugenol. Release studies revealed different profiles, with eugenol showing slower diffusion due to stronger interaction with lignin, while acetyl eugenol was released more rapidly. The incorporation of microparticles into alginate films preserved the structural homogeneity, integrity, and thermal stability. Microparticles and composite films exhibited antimicrobial activity, particularly against *S. aureus*. The composite films maintained measurable inhibition in several formulations, with inhibition zones comparable to those obtained using filter paper discs placed over lignin microparticles loaded with active compounds. This behavior may be associated with the retention of active compounds within the lignin matrix and their gradual diffusion from the alginate polymer network. These findings demonstrate that structural modification of phenolic molecules can be used to tailor their interaction with lignin-based polymeric carriers, providing a simple strategy for controlling encapsulation efficiency, release behavior and antimicrobial performance of sustainable polymer delivery systems.

DECLARATIONS

Author Contribution

L.B.: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing - Original Draft, Visualization; M.E.T.: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing - Original Draft, Visualization, Project administration, Funding acquisition; J.G.: Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing - Original Draft, Visualization; P.G.: Methodology, Validation, Data Curation, Writing - Review & Editing; A.E.A.: Writing - Review & Editing, Supervision; D.E.: Methodology, Data Curation, Writing - Review & Editing, Supervision; C.A.B.: Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

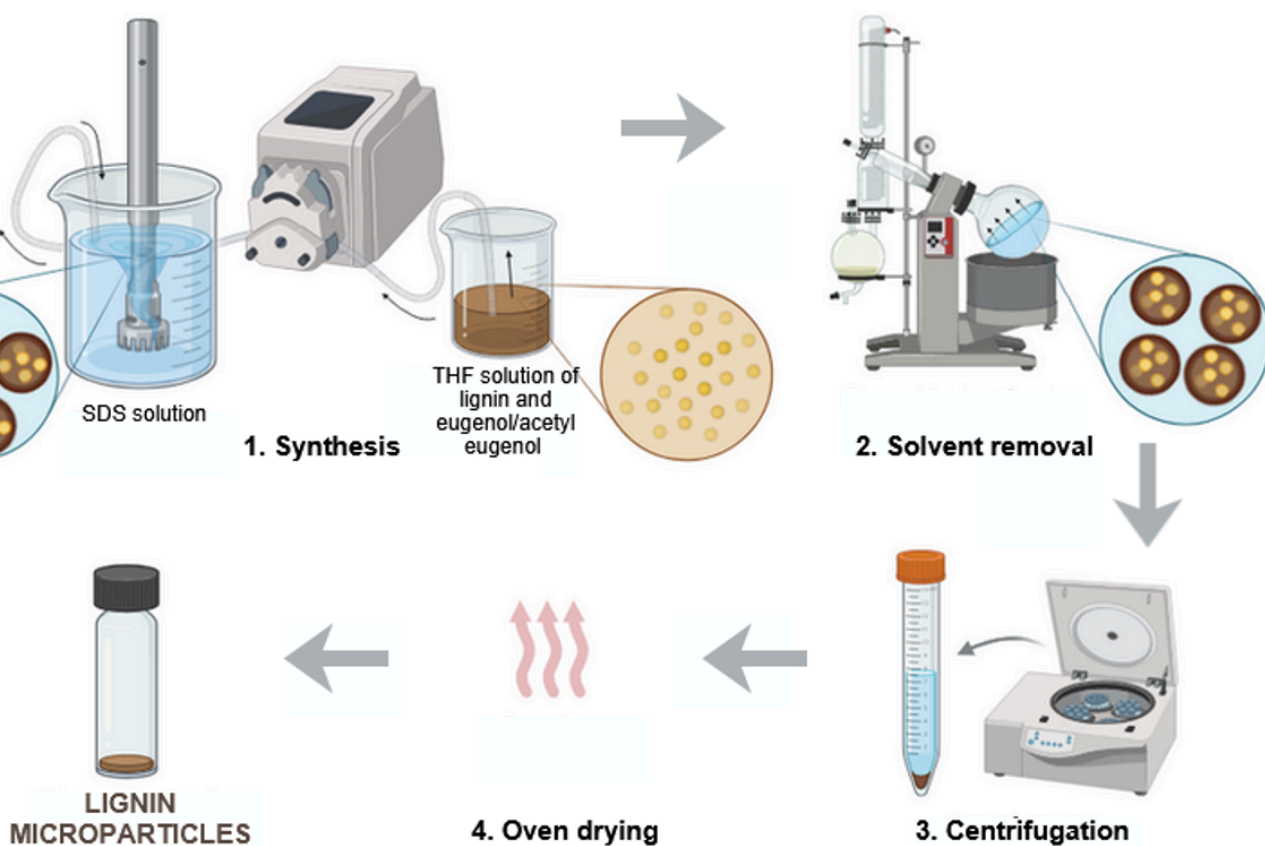


Figure 1

Schematic illustration of the methodology used for the preparation of lignin microparticles loaded with eugenol or acetyl eugenol.

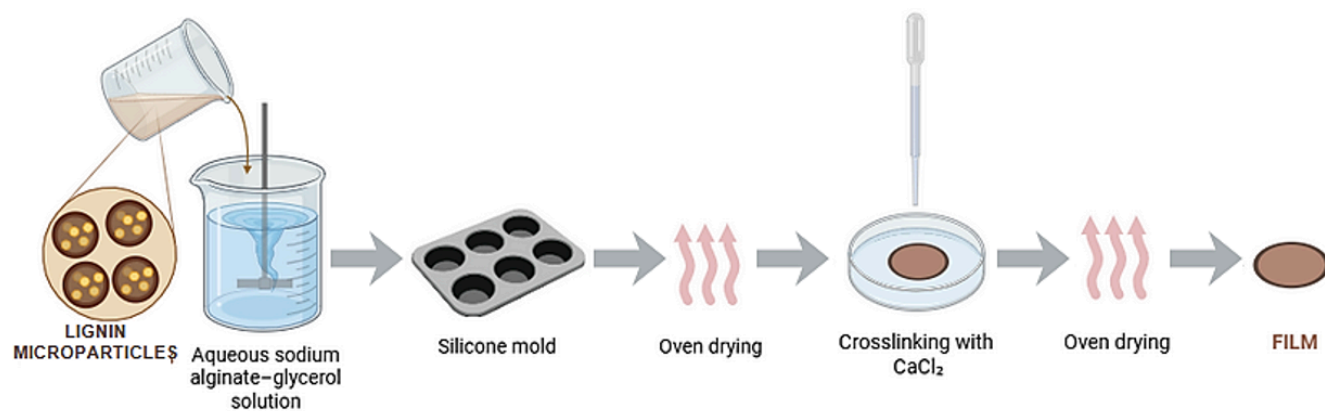


Figure 2

Schematic illustration of the preparation process of sodium alginate films incorporating lignin microparticles loaded with eugenol or acetyl eugenol.

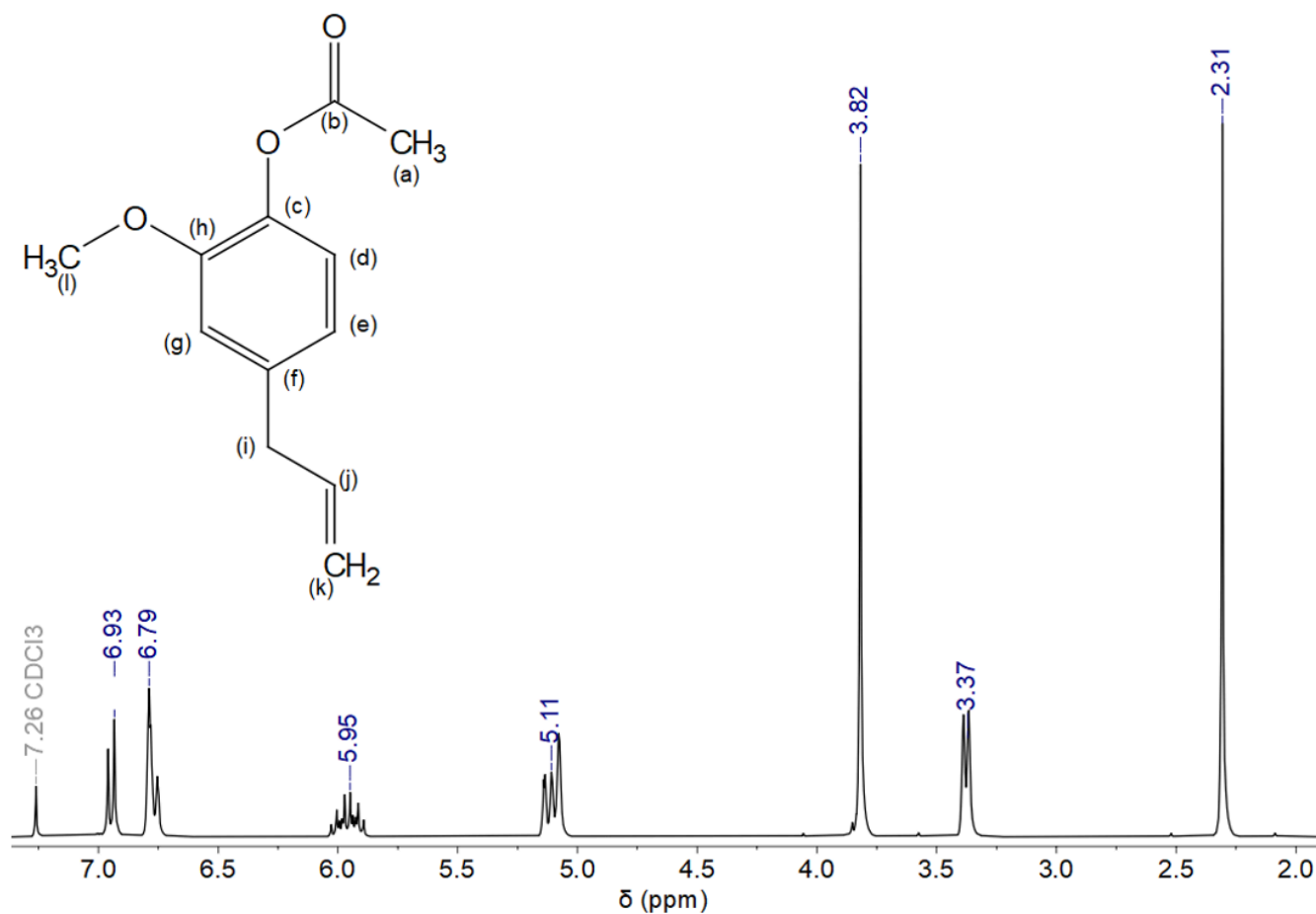
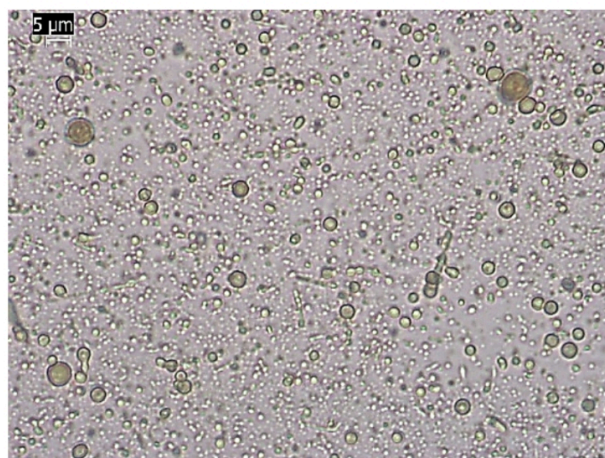


Figure 3

¹H NMR spectrum of the synthesized acetyl eugenol.

a)



b)

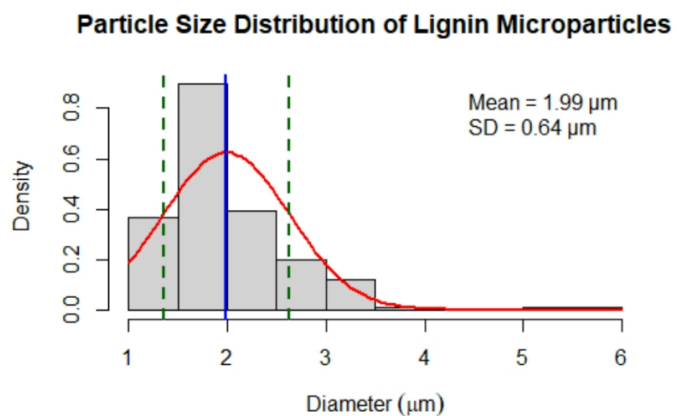


Figure 4

Optical micrograph of ionic lignin microparticles. Scale bar: 5 μm (a). Particle size distribution of lignin microparticles. The red line represents the normal distribution fitted to the data; the blue line indicates the mean diameter and green dashed lines correspond to standard deviation (b).

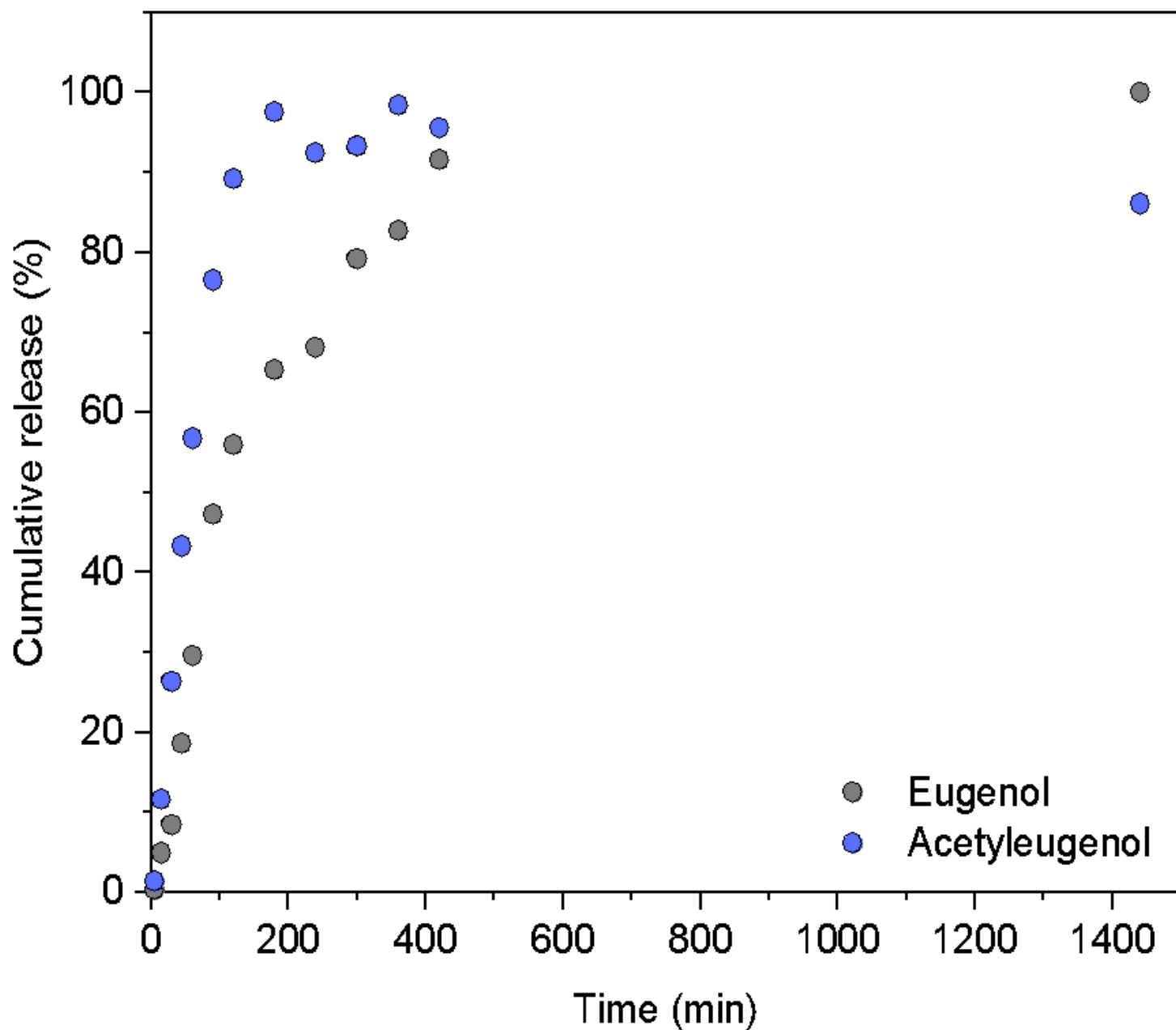
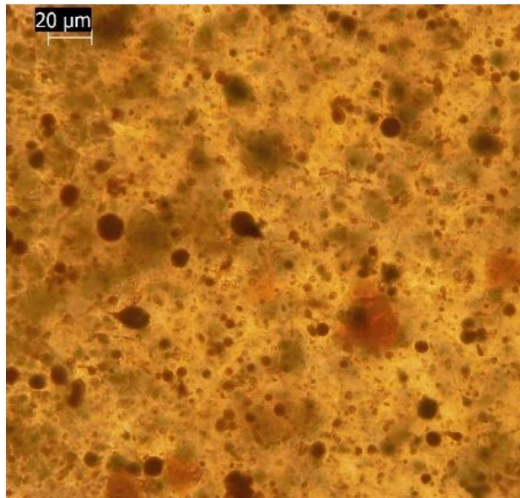


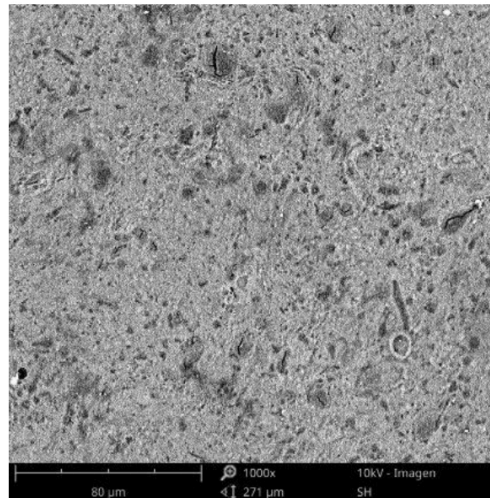
Figure 5

In vitro release profiles of eugenol and acetyl eugenol from lignin microparticles formulated with 20% (w/w) active compound relative to lignin.

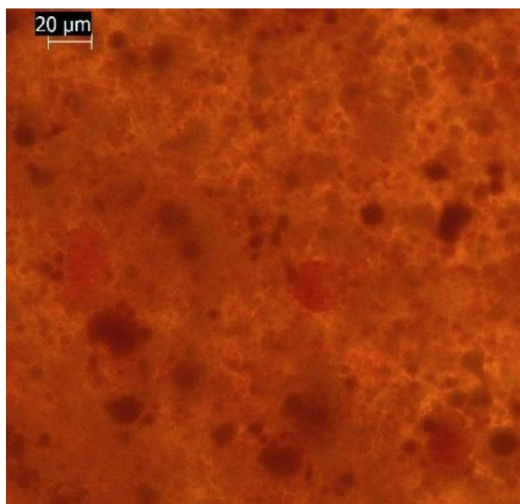
a)



b)



c)



d)

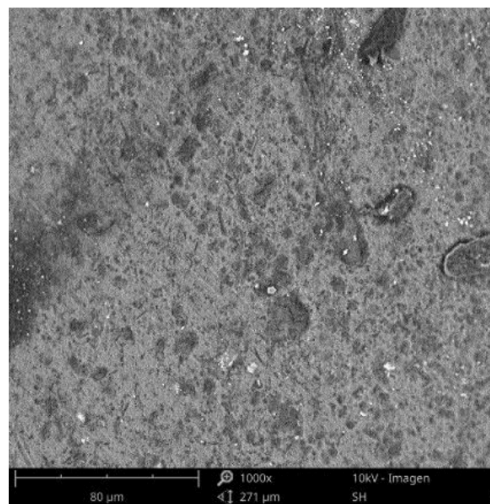


Figure 6

Optical microscopy images (a, c) and SEM micrographs (b, d) of sodium alginate films containing 10 wt% (a, b) and 30 wt% (c, d) of lignin microparticles. Scale bars: 20 μm (a,c) and 80 μm (b,d).

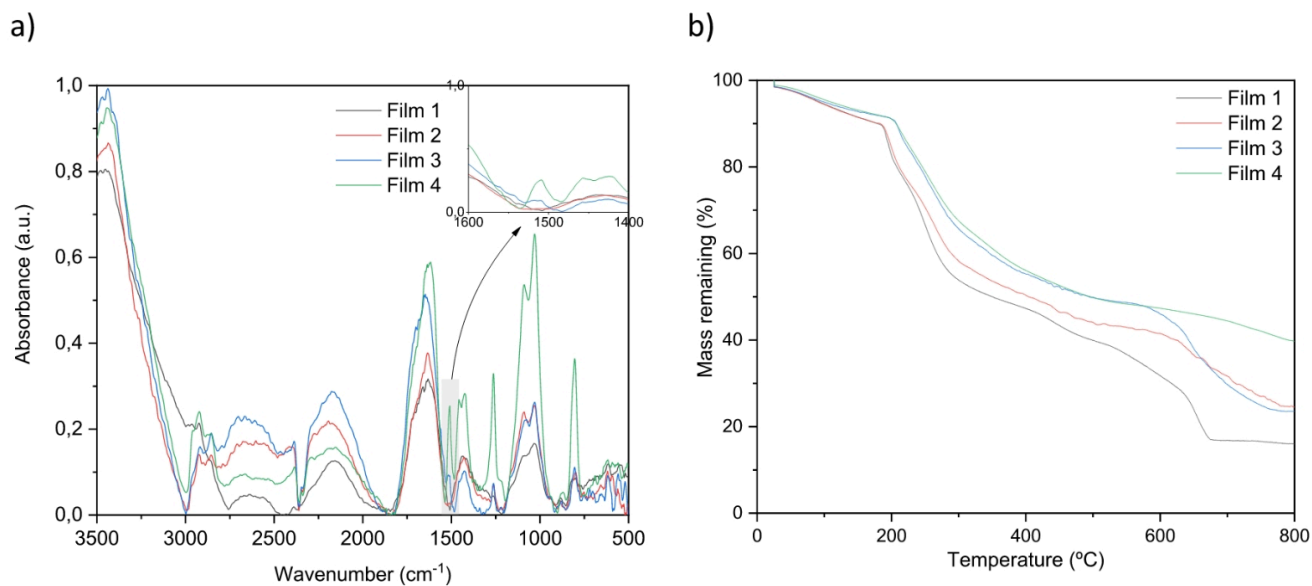


Figure 7

Sodium alginate films containing 0, 10, 20, and 30% lignin particles (Films 1-4, respectively): (a) FTIR spectra showing the characteristic bands of the polymeric matrix and the signals associated with the incorporation of lignin. (b) TGA thermograms illustrate the effect of particle content on the thermal stability and degradation behavior of the composite material.

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