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Microcapsules Containing *Lippia organoides* Kunth Essential Oil as a Natural Alternative to Control Food Spoilage Pathogens: A Preliminary Study

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ABSTRACT

This research presents a preliminary study on the volatile retention and antimicrobial potential of microencapsulated *Lippia organoides* essential oil (LoEO), aiming to analyze the viability of the process in terms of maintaining its main volatile constituents and its effectiveness against food-contaminating bacteria. Microcapsules were obtained by drying in a spray dryer using a mixture of gum arabic (GA) and maltodextrin (MD) as the raw materials for the wall (3:3.6 m/m). The resulting powder was characterized by its morphology, encapsulation efficiency, retention of volatile constituents, and antimicrobial potential. The morphological analysis was performed by scanning electron microscopy (SEM), while the chemical composition was determined by gas chromatography with a flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS). The antimicrobial potential was evaluated using the broth microdilution method. The microcapsules exhibited a wrinkled external morphology without apparent cracks, indicating protection and retention of the core. The encapsulation efficiency was 78%. Chromatographic analysis revealed that the process provided a relative increase in the main constituents (carvacrol and *p*-methoxycarvacrol), resulting in an improvement of the oil's antimicrobial potential. However, due to the exploratory nature of this work, these conclusions should be interpreted as indicative, serving as a foundation for future scale-up and shelf-life studies in food matrices.

1 | Introduction

Microbial action and oxidation reactions are considered the most significant causes of food deterioration. Despite the numerous preservation techniques available, the food industry still needs adequate control over spoilage. Furthermore, consumers' concerns regarding synthetic preservatives have resulted in an increased demand for natural products that are effective and safe for human consumption [1, 2].

Interest in the use of essential oils (EOs) to preserve food quality has increased significantly in recent years. These products are viable alternatives to increase the shelf life of perishable food products, preventing the proliferation of spoilage microorganisms and pathogens [3]. However, EOs are composed of volatile constituents, most of which are bioactive and may undergo degradation when exposed to high temperatures, oxidation, and UV radiation [4]. Furthermore, reactions between EO components and lipids, carbohydrates, and other additives, as well as the

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storage conditions, can reduce the biological activity of antimicrobial constituents [5]. Therefore, these effects can significantly alter the quality of the final products, limiting the use of EOs in commercial applications.

Several methods have been introduced to improve the solubility, stability, and bioavailability of EOs. Microencapsulation is one of the most efficient processes for minimizing damage to these compounds, thereby increasing their chemical stability and enhancing biological efficiency, while facilitating transport and handling through conversion into a powdered state [6, 7].

Among the different techniques for EO microencapsulation, spray drying stands out as a well-established process in many sectors of the food industry [8]. Once encapsulated, the core is protected from external environmental factors and other components present in the food matrix, thereby improving the nutritional value of the food matrix without compromising the food's organoleptic properties. Additionally, this process can increase the shelf life and stability of the active ingredient present in the final product [9].

For instance, a study conducted by Liu et al. [10] demonstrated that the incorporation of EOs into biodegradable films, such as those made from polysaccharides, improves the physical and antimicrobial properties of food packaging.

Lippia origanoides Kunth, known in northern Brazil as “Salvado-marajó” and “Alecrim-d’angola,” is a shrubby species with a perennial life cycle. Its aerial parts are used in cooking as a flavoring for regional dishes, as well as in the treatment of gastrointestinal disorders and respiratory diseases, and as an antiseptic for mouth and throat irritation. This species is called “Oregano” in Mexico and is used as a substitute for *Lippia graveolens* Kunth and *Origanum vulgare* L., both known as Mexican and Greek oreganos, respectively [11–13].

The oil composition of *L. origanoides* has shown significant variation according to its main constituents. It is represented by different chemotypes, as previously described: *p*-cymene, α - and β -phellandrene, and limonene (chemotype A) [14]; carvacrol (chemotype B) [11, 13]; thymol (chemotype C) [14, 15]; 1,8-cineole (chemotype D, syn. *L. schomburgkiana* Schauer) [16]; and, more recently, (E)-methyl cinnamate and (E)-nerolidol (chemotype E) [17], with the occurrence in the Brazilian and Colombian Amazon.

The bioactivity of the EO produced from *L. origanoides* has been evaluated. It showed antimicrobial activity against some pathogenic microorganisms [11, 13, 18–22]; repellent activity against pests living in stored grains, such as *Sitophilus zeamais* and *Tribolium castaneum* [23, 24]; an inhibitory effect on the replication of the yellow fever virus in vitro [25]; antiprotozoal activity against *Leishmania chagasi* and *Trypanosoma cruzi* [26, 27]; and a DNA protective effect against bleomycin-induced genotoxicity [28]. These qualities give the plant a biological potential for its use in controlling some infectious diseases associated with its EO, which possesses important properties of significant interest in the cosmetics and food markets.

In this study, our objective was to evaluate the effects of the microencapsulation process of *Lippia origanoides* essential oil (LoEO) on the retention of its main volatile compounds and to assess its antimicrobial potential against *Bacillus cereus*, *B. subtilis*, and *Salmonella typhimurium*, species often associated with

foodborne diseases. The microcapsules were obtained using the spray drying technique, and the microencapsulated EO was characterized in terms of encapsulation efficiency, chemical composition, and antimicrobial potential.

2 | Materials and Methods

2.1 | Experimental Design

In this study, the experimental design was developed as a technical evaluation focused on the feasibility of the microencapsulation process. Consequently, the analytical scope is strategically centered on the characterization of the resulting particles and the initial validation of their antimicrobial potential.

2.2 | Reagents

Solvents were purchased from Merck (Rio de Janeiro, Brazil). Culture media for microorganisms were obtained from Himedia (Mumbai, India). Drugs and reagents were purchased from Sigma–Aldrich (St. Louis, MO, EUA). Gum arabic (GA) and maltodextrin (MD) were obtained from Vetec (Brazil).

2.3 | Plant Material

Leaves and thin branches of *L. origanoides* were collected in an experimental plantation at the Universidade Federal do Oeste do Pará (UFOPA), located on Rodovia Everaldo Martins (PA-457), km 26, municipality of Santarém, State of Pará, Brazil. The collection site coordinates are 02°30'870" S and 54°56'416" W, with an altitude of 52 m. Specimens of the plant are deposited in the herbarium of the Universidade Federal de Juiz de Fora, Minas Gerais, Brazil, under number CESJ-64029. The EO was obtained by hydrodistillation (3 h) and dried in anhydrous sodium sulfate. Its percentage content was calculated based on the dry weight of the plant. The oil was kept refrigerated (10°C) until its constituents were analyzed.

2.4 | Analysis of the Composition of Free and Microencapsulated EO

The analysis of the EO composition was carried out on a gas chromatography coupled to mass spectrometry (GC–MS) QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan), equipped with an AOC-20i automatic injector and the GC/MSSolution software, a database with Adams [29] and Mondello [30] libraries. An Rxi-5 ms fused silica capillary column (Restek Corporation, Bellefonte, PA, USA) measuring 30 m $\times$ 0.25 mm $\times$ 0.25 μ m (film thickness) was used. The analysis conditions were: injector temperature of 250°C; oven temperature programming from 60 to 240°C (3°C/min); helium as carrier gas, set at a linear velocity of 36.5 cm/s (rate of 1.0 mL/min); injection in split mode (1 μ L); split ratio 1:20; electron impact ionization (EI) of 70 eV; ionization source and transfer line temperatures of 200°C and 250°C, respectively. Mass spectra were obtained by automatic scanning every 0.3 s, with fragment masses in the 35–400 m/z range. The retention index was calculated for all oil constituents using a homologous series of C8–C20 n-alkanes (Sigma–Aldrich), according to the linear equation of Van den Dool and Kratz [31]. The oil constituents were identified by comparing their mass spectra (molecular mass and fragmentation pattern) with the existing

spectra in the GC–MS system libraries and the literature [29, 30] on the basis of their retention indices.

2.5 | Microencapsulation of EO, Yield, Efficiency, and Retained and Surface Oil Content

GA and MD (dextrose equivalent 4/7) were used as wall materials (3:3.6 m/m, 6.6%) [32]. The concentration of EO added was 1%, based on the total volume of the emulsion. The wall material was previously dissolved in deionized water and maintained for 12 h at room temperature to ensure complete saturation of the polymer molecules. Subsequently, the oil was slowly added to the dispersion, under stirring (10,000 rpm) for 10 min, using a high-speed digital homogenization system (Ultra Turrax – T25, Wilmington, NC, USA) [33–35]. The drying process was carried out in a spray dryer equipment (model MSD 1.0, LABMAQ, Ribeirão Preto, Brazil), equipped with a cylindrical chamber 50 cm high by 9 cm in diameter, with a double fluid atomizing nozzle and opening 0.7 mm in diameter. The feeding was carried out by a peristaltic pump, with a spray flow of 45 L/min, inlet air temperature of 160°C and outlet air temperature of 124°C, drying airflow at a speed of 4 mL/min, and the airflow volume of 4 m³/min. The compressed air pressure for the atomization flow was set at 60 psi. The feed emulsion was continuously homogenized using a magnetic bar stirring system during the process. The resulting product was stored in an amber glass container, protected from light and at a temperature of 4°C.

The percentage yield of microcapsules was determined by the ratio between the total mass of microcapsules and the total mass of solids (polymers + EO) used in preparing the emulsion, according to Banerjee et al. [36]. The encapsulation efficiency of EO was calculated, considering the retained oil content and the surface oil content in the microcapsules [37]. Briefly, 10 mg of microcapsules were dissolved in 0.5 mL of distilled water and homogenized for 1 min, followed by oil extraction by adding 10 mL of acetone. After mixing under vortex agitation, the samples were centrifuged at 3000 rpm for 10 min. Using a previously prepared standard curve, the external normalization method determined the oil content (µg/mL) extracted in the liquid phase. The surface oil content on the outside of the particle (µg/mL) was calculated according to Delfiya et al. [37], with some modifications. Briefly, 5 mL of ethanol was added to 1 g of microcapsules, manually shaken for 30 s, and then centrifuged at 3000 rpm for 10 min. After phase separation, the supernatant was filtered through a Nylon filter (0.22 µm pore size (Allcrom, Brazil), and the surface oil was quantified using a previously prepared standard curve.

To plot the standard curves, solutions containing different EO concentrations (0.2 to 3.0 µg/mL) were prepared. Absorbance values were determined using a spectrophotometer (UV–Vis, Quimis, Brazil) at 400 nm. The calibration curve was obtained by plotting the standard concentrations of the EO (x) and the absorbance values (y), receiving the equation: $y = 0.0514x - 0.0164$, where y and x represent the absorbance values and the concentration of the EO, respectively ($R^2 = 0.9755$).

2.6 | Morphological Analysis

For morphological analysis, the microcapsules that contained EO were attached to metallic adhesive tapes adhered to metallic stubs. A fine layer of gold was deposited onto the stubs using

an evaporator (Denton Vacuum – Desk V). The observations were made using a JEOL1 model JSM-6610 scanning electron microscope (SEM) equipped with an EDS accessory (Thermo Scientific NSS Spectral Imaging), operated with a voltage of 5 kV [32]. The images were captured after 3500x magnification.

2.7 | Analysis of the Antimicrobial Action of Microencapsulated EO Against Food-Contaminating Bacteria

Strains of *Bacillus subtilis* (CCCD B005), *Bacillus cereus* (CCCD B001), and *Salmonella typhimurium* (CCCD S004) were commercially purchased in lyophilized form and hydrated in nutrient broth at $37 \pm 1^\circ\text{C}$ for 24 h. Inocula were prepared by directly inoculating colonies in 1 mL of sterile saline and adjusted to standard 0.5 on the McFarland scale, corresponding to 1.5×10^8 CFU/mL.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of microencapsulated EO capable of inhibiting the growth of microorganisms. It was determined by the standard broth microdilution method [38] with modifications. Briefly, 40 mg of microcapsules were diluted in 1 mL of sterile distilled water. The mixture was vortexed for ~2 min. with subsequent centrifugation at 3000 rpm for 10 min, to release the microencapsulated oil. Based on this solution, aliquots with concentrations ranging between 0.007 and 20 mg/mL were also prepared. The free EO was evaluated in concentrations ranging between 5 and 0.02 mg/mL. The inocula were adjusted to standard 0.5 on the McFarland scale and diluted in sterile saline solution (1:10) to obtain a final concentration of 1.5×10^4 CFU/mL. The tests were carried out in 96-well plates, each receiving 90 µL of the specific concentration of solutions containing the microencapsulated oil plus 90 µL of sterile Mueller Hinton Broth (MHB) and 20 µL of the inoculum. Ampicillin was used as a positive control at concentrations ranging from 0.1 to 46.9 µg/mL to validate the experiment. The action of the wall materials control and the sterility of the medium control were carried out simultaneously. Each well contained a final volume of 200 µL. Inhibition of bacterial growth was revealed by adding sterile resazurin solution (20 µL, 0.02%), followed by re-incubation for 3 h under the abovementioned conditions. Tests were performed in triplicate.

2.8 | Statistical Analysis

All data were subjected to analysis of variance (ANOVA). Inferential statistics were applied to determine significant differences between means using the Tukey test ($p \leq 0.05$), with a confidence interval established at 95%.

3 | Results and Discussion

3.1 | EO Composition

It is known that the biological potential of a given EO changes depending on its composition. Hence, maintaining EO composition and its bioactive constituents during the post-microencapsulation process is as important as the encapsulation efficiency itself. In this work, the relative percentages of the constituents present in the LoEO, before and after the microencapsulation process, were determined. The chemical composition and respective percentages are shown in Table 1.

TABLE 1 | *Lippia origanoides* essential oil: chemical composition before and after the microencapsulation via spray drying.

Constituents (%)	Essential oil (%)			
	RI _{Calc}	RI _{Lit}	Free	Micro-encapsulated
(3Z)-Hexenol	850	850	0.1	—
α-Thujene	927	924	0.9	—
α-Pinene	934	932	0.5	—
1-Octen-3-ol	976	974	0.2	—
β-Pinene	978	974	0.2	—
Myrcene	991	988	1.4	0.5
α-Terpinene	1017	1014	0.9	—
p-Cymene	1025	1020	10.1	7.0
Limonene	1029	1024	0.2	—
1,8-Cineole	1032	1026	0.9	—
γ-Terpinene	1059	1054	2.7	—
cis-Sabinene hydrate	1068	1065	0.1	—
Linalool	1100	1095	5.0	2.9
Ipsdienol	1146	1140	0.3	—
Borneol	1167	1169	0.1	—
Umbellulone	1175	1167	0.3	—
Terpinen-4-ol	1179	1174	1.0	—
p-Cymen-8-ol	1186	1179	0.1	—
α-Terpineol	1192	1186	0.1	—
Thymol methyl ether	1235	1232	0.5	0.5
Carvacrol methyl ether	1238	1241	0.4	—
Geranial	1268	1264	0.1	—
Thymol	1293	1289	10.0	8.0
Carvacrol	1305	1298	36.7	57.7
Thymol acetate	1355	1349	0.6	—
Carvacrol acetate	1373	1370	1.5	—
Geranyl acetate	1384	1379	0.4	—
(E)-Caryophyllene	1424	1417	3.6	3.6
trans-α-Bergamotene	1438	1432	0.3	—
α-Humulene	1458	1452	0.5	—
γ-Murolene	1480	1478	0.1	—
p-methoxythymol (3-hydroxy-6-methoxy-p-cymene)	1485	1482	0.4	—
β-Selinene	1490	1489	0.2	—
p-methoxycarvacrol (2-hydroxy-5-methoxy-p-cymene)	1495	1491	9.8	15.6
(Z)-α-Bisabolene	1505	1506	0.2	—

(Continues)

TABLE 1 | (Continued)

Constituents (%)	Essential oil (%)			
	RI _{Calc}	RI _{Lit}	Free	Micro-encapsulated
β-Bisabolene	1511	1505	0.4	—
γ-Cadinene	1518	1513	0.1	—
δ-Cadinene	1527	1522	0.3	—
(E)-α-Bisabolene	1545	1540	0.7	—
Caryophyllene oxide	1587	1582	2.9	1.8
Humulene epoxide II	1614	1608	0.2	—
1,10-di-epi-Cubenol	1619	1619	0.1	—
α-Murolol	1645	1644	0.1	—
α-cadinol	1658	1652	0.2	—
α-Bisabolol	1686	1685	0.1	—
Unidentified constituents	—	—	1.7	—
Total (%)	—	—	97.2	97.6

Note: RI_{Calc} = Retention index on Rxi-5 ms Column; RI_{Lit} = Retention index of literature [29].

The primary constituents of the *L. origanoides* free EO consisted of carvacrol (36.7%), p-methoxycarvacrol (9.8%), thymol (10.0%), and p-cymene (10.1%). The microencapsulation process led to a redistribution of the chemical profile, increasing the percentages of carvacrol (57.7%) and p-methoxycarvacrol (15.6%), while the levels of thymol and p-cymene declined to 8.0% and 7.0%, respectively.

Additionally, minor constituents such as linalool and myrcene showed slight reductions, shifting from 5.0% to 2.9% and from 1.4% to 0.5%, respectively. This compositional reorganization can be attributed to three main factors. First, the chemical affinity and intermolecular interactions between the core and the wall material played a decisive role. The significant retention of carvacrol and its oxygenated derivatives can be explained by the presence of hydroxyl groups, which facilitate the formation of hydrogen bonds with specific sites on the polysaccharide and glycoprotein structures of the GA matrix [39, 40], leading to a higher relative concentration of these bioactive markers in the final microencapsulated oil. Consequently, volatile constituents lacking such functional groups, or those with lower affinity for the polymers, exhibit lower relative retention.

Second, the molecular geometry and diffusion kinetics within the matrix influenced the retention of specific monoterpenes. According to Bertolini et al. [41], the diffusion of compounds through the wall material is determined not only by molar volume but also by molecular diameter. Therefore, monoterpenes with similar molecular weights and solubility may exhibit variations in retention due to structural differences, which dictate their ability to migrate through the matrix before the formation of the protective crust.

Finally, the thermal stability and selective evaporation during the process explain the proportional increase of the more stable compounds. High drying temperatures promote the selective evaporation of low-molecular-weight molecules with high volatility

and greater water solubility, which are lost to a higher degree during the process [40, 42]. The thermal loss of these less stable constituents, such as α -cymene and myrcene, results in a proportional increase in the remaining, more stable phenolic compounds, such as carvacrol [43].

While GC–MS is the gold standard for EO characterization, it is essential to acknowledge that the method's sensitivity to highly volatile compounds and potential mass discrimination in the injector may influence the detection of minor constituents. However, by maintaining strictly identical analytical conditions for both free and encapsulated oils, the observed shifts in the relative profile, such as carvacrol enrichment, can be reliably attributed to the thermodynamic and kinetic phenomena of the microencapsulation process, thereby minimizing analytical bias.

3.2 | Percentage Yield and Encapsulation Efficiency

Under the experimental conditions of this study, the spray drying process achieved a yield of 30.3%. When compared to the literature, this value is higher than the 24.4% yield reported by Fernandes et al. [8, 44] for the same species using varying proportions of GA alone, and slightly lower than the 39.5% obtained by Teodoro et al. [7] with a modified starch/MD blend. The low yields in laboratory-scale spray drying are primarily attributed to the high surface-area-to-volume ratio of the equipment, which promotes wall deposition. Furthermore, the 'stickiness' of particles during the transition to a glassy state favors the formation of strong liquid and solid bridges with the drying chamber, hindering the re-entrainment of the powder [45].

The use of different polymeric matrices for the microencapsulation of antimicrobial agents, such as thymol and carvacrol, results in distinct controlled release profiles. These variations in release kinetics are attributed to the specific physicochemical properties of each wall material and their molecular interactions with the encapsulated compounds [46]. According to Delfiya et al. [37], higher concentrations of GA increase emulsion viscosity, which serves as a kinetic barrier that restricts droplet movement and suppresses coalescence. From the perspective of emulsion stability theory, this effect is primarily driven by the amphiphilic nature of GA proteinaceous fraction, which adsorbs at the oil–water interface to provide robust steric stabilization. This prevents droplet coalescence through physical repulsion between polymer chains, maintaining a fine droplet size during atomization [47].

Similarly, Jafari et al. [40] and Mehran et al. [48] emphasize that a high solids content reinforces this viscous framework, facilitating the transition of the polymer matrix into a glassy state. This accelerates the development of a resilient solid crust, a mechanism central to the Selective Diffusion Theory. In this state, the polymer matrix becomes a selective barrier that significantly reduces the diffusion coefficient of carvacrol while allowing the preferential evaporation of water, thus maximizing EE.

In a study involving the EO of *Lippia sidoides*, the EE was proportionally linked to the increase in GA content within the MD mixture. This effect was attributed to the superior film-forming and emulsifying properties of GA. While MD exhibits limited emulsifying and retention capacity even at high concentrations,

GA stands out as an efficient encapsulating agent for volatile compounds, providing superior retention after the drying process [49]. Consequently, the blend and proportion of GA and MD utilized in this work demonstrated the necessary resilience to withstand the specific thermal and mechanical conditions of the encapsulation process.

Spray drying technology can be considered a suitable approach for the microencapsulation of LoEO, offering scalability, operational flexibility, and cost-effectiveness. This method is particularly efficient in producing particles with low water activity, which not only enhances microbiological stability and extends shelf life but also ensures a controlled and predictable release of the encapsulated bioactives [50].

Although efficient, the method imposes strict criteria regarding the solubility of wall materials and presents limitations in processing thermolabile substances, in addition to the inherent thermal energy consumption of the operation. Despite these challenges, spray drying demonstrates superior efficiency compared to freeze-drying for oil encapsulation, establishing itself as a significantly more cost-effective alternative, with operational costs estimated to be 30–50 times lower [51].

Furthermore, in comparison to the microencapsulation technique by emulsification and coating described by Guarda et al. [52], it is observed that both methodologies demonstrate high efficiency in the retention of volatile compounds, albeit with distinct purposes. While Guarda et al. [52] utilized a GA emulsion processed at room temperature for direct application onto films, the spray drying technique employed in this work proved equally efficient in protecting these compounds, even under the thermal stress inherent to the process.

In comparison to the complex coacervation method proposed by Muneratto et al. [53] and Sun et al. [54], which relies on electrostatic interactions between proteins and polysaccharides to envelop carvacrol in a dense hydrated matrix, our spray drying approach achieved comparable retention performance. This demonstrates that the rapid formation of a solid crust during atomization effectively seals the carvacrol within the particles, matching the capabilities of phase-separation methods while providing the advantage of a stable, dry powdered ingredient.

Although release kinetics were not experimentally determined in this work, the observed structural integrity and matrix composition suggest that the Peppas–Sahlin model could be a highly effective framework for future studies. This model would enable a precise distinction between Fickian diffusion and polymer chain relaxation as the driving forces behind the bioactive release [55], further supporting the industrial potential of these microcapsules.

3.3 | Morphological Characterization

The morphology of the microcapsules was evaluated by SEM, allowing the external structure to be visualized and its integrity assessed for the presence of pores or cracks. The effect of the spray drying process on the resulting morphology is shown in Figure 1.

The microcapsules were characterized as predominantly spherical particles of varying sizes, exhibiting a wrinkled external

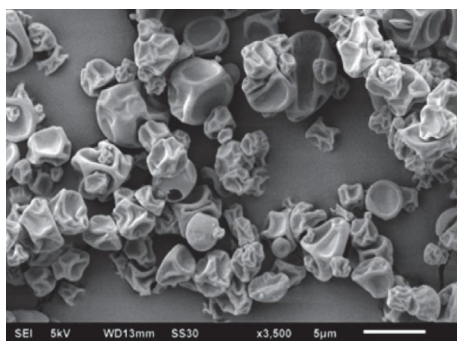


FIGURE 1 | Morphological analysis of microcapsules containing *L. origanoides* essential oil. Images captured by electron microscopy at 3500x magnification.

morphology with significant surface indentations (shriveling). The absence of apparent cracks or fissures across the samples indicates the successful retention of the core material and the structural integrity of the polymer matrix.

These morphological traits, including the “crater-like” depressions observed, align with findings reported for other EOs encapsulated with GA and MD blends [49, 56]. Specifically, Krishnan et al. [49] observed that GA-based microcapsules were partially spherical but exhibited significant surface denting; similarly, Botrel et al. [56] noted that particles encapsulated with MD, GA, and modified starch displayed rounded shapes with crater-like depressions.

The irregular morphology of the microcapsules is due to the rapid solidification of the crust during drying, a process that induces contraction during the initial evaporation stage [57]. Particularly in smaller particles, the formation of deep grooves indicates that wall hardening occurred before the completion of internal expansion, a manifestation of the structural integrity of the matrix [56].

Although release kinetics were not experimentally evaluated in this study, the observed polydispersity and particle size distribution suggest a potential multi-stage release profile. As described by Gallo et al. [58], smaller particles, due to their higher surface-to-volume ratio, likely facilitate an initial release governed by Fickian diffusion, while larger, denser structures are expected to provide sustained protection and prolonged release.

Therefore, the observed morphology is not merely an esthetic byproduct of the process, but a structural determinant. These findings suggest that the LoEO is likely to remain chemically protected against environmental pro-oxidants during storage, while the physical properties of the encapsulating matrix

modulate its release profile. Furthermore, from an industrial perspective, the predominantly spherical shape and structural rigidity of these particles are expected to ensure adequate powder flowability and bulk density. Such traits facilitate mechanical handling, storage, and uniform integration into food matrices, thereby minimizing the risk of premature core release during industrial processing steps.

3.4 | Antimicrobial Activity of Microcapsules Containing LoEO

The antimicrobial potential of LoEO has been previously reported [11, 18, 59]. In the present study, even after the spray drying microencapsulation process, the oil retained its antimicrobial activity, which is attributed to the maintenance of phenolic compounds at higher concentrations relative to the other constituents (Table 2).

This successful maintenance of bioactivity holds important implications for the food industry, particularly for the development of functional ingredients and the stabilization of volatile compounds in complex industrial matrices. This aligns with recent advancements in the field, where micro- and nanoencapsulation of bioactive EOs is increasingly recognized as a pivotal strategy for biotechnological and biomedical applications, offering enhanced controlled release and improved therapeutic or preservative efficacy [60].

Regarding the DD assay, the free EO exhibited inhibition zones ranging from 25.8 ± 0.6 mm to >40 mm, whereas the microencapsulated oil yielded significantly lower diameter values (ranging from 12.9 ± 0.5 mm to 22 ± 0.6 mm; $p \leq 0.05$). This reduction in the inhibition zone reflects the typical behavior of controlled-release systems, in which the polymer matrix modulates the diffusion of volatiles; although this limits the immediate bioavailability of the compounds, it ensures a gradual and sustained release into the solid medium [61].

Despite the lower DD values compared to the free oil, the microcapsules maintained relevant inhibitory activity against both Gram-positive and Gram-negative bacteria, confirming that the spray-drying process preserved the bioactive integrity of the carvacrol-rich oil.

Regarding the MIC, both free and microencapsulated LoEO exhibited relevant antibacterial activity against the strains tested. Notably, the microencapsulation process was associated with a discrete reduction in MIC values, from 0.62 to 0.4 mg/mL, against *B. cereus* and *S. typhimurium*. More significantly, a six-fold decrease in the MIC value against *B. subtilis* was observed following encapsulation.

TABLE 2 | Antimicrobial activity of free and microencapsulated *Lippia origanoides* essential oil against food-borne microorganisms.

Microorganisms	Free Oil		Microencapsulated oil		Ampicillin	
	DD (mm)	MIC (mg/mL)	DD (mm)	MIC (mg/mL)	DD (mm)	MIC (µg/mL)
<i>Bacillus cereus</i> CCCD B001	>40	0.62	$22 \pm 0.6^*$	0.4	24.2 ± 0.3	1.5
<i>Bacillus subtilis</i> CCCD B005	37.4 ± 0.4	1.25	$16 \pm 0.8^*$	0.2	22.4 ± 0.6	0.2
<i>Salmonella typhimurium</i> CCCD S004	25.8 ± 0.6	0.62	$12.9 \pm 0.5^*$	0.4	18.9 ± 0.2	1.5

Abbreviations: DD, disk diffusion; MIC, minimum inhibitory concentration.

*Indicates a significant difference between the free oil and microencapsulated oil groups according to Tukey's test ($p \leq 0.05$).

This enhanced inhibitory profile suggests that the polymer matrix preserved the bioactivity of the volatile compounds, possibly facilitating their interaction with the bacterial cell wall. Furthermore, this trend may be correlated with the proportional increase in carvacrol content post-processing, a constituent recognized as the primary driver of the antimicrobial properties of LoEO.

As demonstrated in a different context by Arana-Sánchez et al. [62], the higher the carvacrol and thymol content in an EO's composition, the greater its inhibitory effect on microbial growth. In accordance with this, our results showed that the percentage of thymol and carvacrol in the microcapsules increased by ~20% compared to the free oil, indicating that the process effectively concentrated the primary active constituents of LoEO.

These findings are consistent with previous studies where encapsulation enhanced the antimicrobial potential of EOs. For instance, the spray drying microencapsulation of *Lippia graveolens* and *Origanum vulgare* oils was found to enhance the efficacy of carvacrol and thymol against pathogenic microorganisms [63]. Similarly, the antimicrobial activity of *Origanum dictamnus* liposomal microcapsules was effectively preserved following the encapsulation process [6].

The increased efficiency in bacterial inhibition observed with microencapsulated EOs can also be attributed to improved solubility and dispersibility, which facilitate interaction with microorganisms and intensify their antimicrobial action [63, 64]. Furthermore, the encapsulation process influences the physicochemical and biological properties of these molecules, inducing structural or stability changes that enhance their overall bioactivity [65].

As synthesized by Sarrazin et al. [66], the mechanism of action of these phenols is based on their lipophilic nature, which allows these monoterpenes to penetrate the lipid bilayer, resulting in membrane disruption, the release of lipopolysaccharides (LPS) in Gram-negative bacteria, and eventual cell death. Additionally, the presence of *p*-cymene aids in this process; although it possesses lower isolated biological activity, its high affinity for biological membranes facilitates the transport and penetration of phenolic compounds into cytoplasmic targets, thereby maximizing the biological efficacy of the encapsulated EO.

Other compounds also detected in our microencapsulated oil, such as α -pinene and β -pinene, affect the integrity of the cytoplasmic membrane, impairing electron transport. Compounds such as linalool and terpineol can denature proteins or act as solvents, enhancing the antimicrobial effect of these oils [67]. Thus, the synergy between these components, successfully preserved by the spray drying process, potentiates the antimicrobial efficacy of the final product.

Furthermore, the observed reductions in MIC values are highly relevant within the context of food safety standards. Lowering the inhibitory threshold allows the food industry to use lower dosages of the antimicrobial agent while maintaining efficacy against target pathogens. This is critical not only to ensure microbiological safety but also to mitigate the sensory impact of EOs, as their intense aroma often limits their application at higher concentrations [68, 69].

The use of *L. origanoides* microcapsules addresses the growing concern over antimicrobial resistance. Unlike single-target

synthetic preservatives, the multi-component nature of this microencapsulated EO exerts pressure on multiple cellular targets simultaneously. This multi-target effect occurs when different compounds synergistically attack various bacterial sites, while release kinetics and physicochemical interactions, such as the enhanced solubility and dispersibility provided by the microencapsulation process, further intensify the antimicrobial delivery and efficacy. This multi-hit mechanism significantly reduces the likelihood of target microorganisms developing resistance through genetic mutations, as they would need to adapt to several metabolic disruptions at once [70]. Furthermore, the controlled release provided by the encapsulation matrix minimizes the risk of sublethal exposure of the bacteria, which is often a trigger for the selection of resistant strains in food environments.

Nonetheless, despite these promising results, the industrial application of EO microcapsules faces significant hurdles. The inherent chemical variability of these compounds, dictated by genetic factors, geographical origin, and extraction techniques, challenges industrial reproducibility. Such compositional instability complicates the standardization required to comply with rigorous regulatory and biological safety standards [71, 72].

Thus, although encapsulation helps mask the strong aroma of carvacrol, sensory studies in specific food matrices are essential to ensure the organoleptic neutrality of LoEO microcapsules. Finally, scaling up the spray drying process from laboratory to industrial levels demands careful optimization due to differences in evaporative rate, throughput, and the resulting physicochemical characteristics of the particles [73].

4 | Conclusion

Microencapsulation by spray drying preserved the main volatile constituents (carvacrol and *p*-methoxy carvacrol) of LoEO, which was associated with an observed increase in antimicrobial activity against the foodborne bacteria tested. These results indicate the technical feasibility of the process under the conditions evaluated. Given that microcapsule properties, such as morphology, yield, and release behavior, depend on process variables, further studies incorporating additional analytical parameters are necessary to fully these observations and optimize the biotechnological application of this species.

Author Contributions

Sandra Layse Ferreira Sarrazin and Ricardo Bezerra de Oliveira: conceptualization. **Sandra Layse Ferreira Sarrazin, Ricardo Bezerra de Oliveira, and Leomara Andrade da Silva:** methodology. **Sandra Layse Ferreira Sarrazin, José Guilherme Soares Maia, Rosa Helena Veras Mourão, and Ricardo Bezerra de Oliveira:** formal analysis, writing – original draft. **Sandra Layse Ferreira Sarrazin and José Guilherme Soares Maia:** review & editing.

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Disclosure

All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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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