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Effect of Microencapsulation on Chemical Composition and Antimicrobial, Antioxidant and Cytotoxic Properties of Lemongrass (*Cymbopogon flexuosus*) Essential Oil

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SUMMARY

Research background. Lemongrass (*Cymbopogon flexuosus*) essential oil (LEO) exhibits antimicrobial and antioxidant properties due to the presence of α-citral and β-citral. Essential oils (EOs) are susceptible to volatilization and oxidation when applied to food matrices. Therefore, a barrier is needed to protect this material. The present study aimed to produce microparticles containing LEO, using Arabic gum (AG) and maltodextrin (MD) through the spray drying technology.

Experimental approach. LEO was extracted by the hydro-distillation method and later microencapsulated with different wall materials. Free and microencapsulated LEO were evaluated for their cytotoxic activity (*Artemia salina*), chemical composition (GC-MS), encapsulation efficiency,

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antioxidant activity (DPPH, ABTS, and FRAP), antimicrobial activity and minimum inhibitory concentration.

Results and conclusions. LEO had a lethal concentration (LC_{50}) of 8.43 µg/mL against *Artemia salina* in the cytotoxic test; a high activity that can be associated with the presence of α-citral (~ 33 %) and β-citral (~ 21 %) in the samples, since these compounds were the main ones that presented bioactive properties. The highest value of microencapsulation efficiency (88.11 %) was obtained when only AG was used as wall material. In general, the microparticles showed satisfactory antioxidant activity (between 348.66 and 2042.30 µmol TE/100g) and bactericidal effect, in vitro, against Gram-positive and Gram-negative microorganisms. In conclusion, the microencapsulated lemongrass essential oils are a promising functional additive to be used in the food and pharmaceutical industries.

Novelty and scientific contribution. This study shows that microparticles containing LEO can be prepared using AG and MD as wall materials via the spray drying process, resulting in high microencapsulation efficiency. The drying process maintained the essential oil's antimicrobial and antioxidant properties. Therefore, the microencapsulated LEO is considered a natural, functional, and a promising additive to be incorporated in the food industry. This material may have an application in several food products due to the investigated and proven potential addressed in this study. Its antimicrobial action can increase the shelf life of fresh and semi-fresh products such as cheese, yogurts, and meat products. In addition, its antioxidant action can delay the lipid and protein oxidation in food products.

Keywords: bioactive properties; arabic gum; maltodextrin; oil retention; spray drying

INTRODUCTION

Lemongrass (*Cymbopogon flexuosus*) is a plant that belongs to the Poaceae family, and it is native to southern Asia and Australia. It is also cultivated in Brazil, commonly found in the states of São Paulo, Minas Gerais, Pará, Pernambuco, Maranhão, Bahia, and Rio de Janeiro (*1*). Lemongrass essential oil (LEO) is extracted from the leaves of the plant *Cymbopogon flexuosus* and has been recognized for presenting strong antimicrobial, antifungal, and antioxidant capacities (*1,2*).

The antimicrobial capacity of LEO is assigned to the main compounds present in its composition, which are α-Citral and β-Citral (*3,4*). According to Balti *et al*. (*5*), citral is used in the food industry as food flavoring for beverages, sweets, frozen dairy desserts, baked foods, gelatins, puddings, and others. It is also used in cosmetics and perfumery for its lemon scent. However, there are some limitations to the direct application of essential oils (EOs) rich in bioactive compounds, due

to the oils' low stability in food or pharmaceutical products, which is influenced by solvents, pH, temperature, oxygen, light, and enzymes (*6*).

Microencapsulation is an alternative process to increase the stability of EOs, preserving their biological compounds of interest. In general, the technique is based on the formation of emulsion droplets containing the essential oil (EO) coated or incorporated into a homogeneous or heterogeneous matrix, producing small microparticles (*7*).

One of the main benefits of microencapsulation is the increase in the stability of essential oils, which consists in the formation of a multicomponent structure in the form of microparticles that are composed of a core material and the encapsulant (also called wall material). The main advantage of this process is that it allows sensitive ingredients to be physically trapped in a matrix, therefore, protecting the encapsulated component. In the case of essential oils, this mechanism will allow the preservation of the compounds present in the oil, responsible for the oil's bioactive properties (*8*).

Spray drying is considered one of the most common methods used to obtain microparticles from a variety of natural raw materials (*9*). The method is relatively easy to execute, few steps are required to obtain a dry product, and it does not require the use of organic solvents, contributing to a satisfactory cost-benefit. The spray drying process has been widely used in food and pharmaceutical processing, aiming to control the release of bioactive compounds (*10,11*). Arabic Gum (AG) and Maltodextrin (MD) are encapsulating materials generally used in the spray drying process, mainly due to their high solubility and low viscosity, which makes them easier to manage (*12*).

In this study, we chose not to use only Maltodextrin in the formulations, because despite being a wall material commonly used in spray drying processes in the industry, due to its low cost, this material presents low compound retention and reduced emulsification capacity. On the other hand, this material is used as a wall material to microencapsulate essential oils due to its good oxygen barrier properties. However, to improve the physical and chemical properties of the microcapsules, high molecular weight materials in the emulsion, such as Arabic Gum, need to be added. In this context, this work aims to develop and characterize the spray dried microparticles produced with AG and a mixture of AG/MD as an alternative to improve the stability of the bioactive compounds present in the valuable LEO.

MATERIALS AND METHODS

Materials

Arabic Gum (Metaquímica Produtos LTDA, Santa Catarina, Brazil) and Maltodextrin (MD, DE 9–12) (Agro-Industry Commercial Cassava SA, Santa Catarina, Brazil) were used as wall materials to formulate the microparticles. Gallic acid, DPPH• (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azino-

bis(3-ethylbenzothiazoline-6-sulfonic acid)); TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were obtained from Sigma-Aldrich (Sigma Co., USA). The Folin-Ciocateau reagent was supplied by Merck. Ferric chloride hexahydrate ($FeCl_3.6H_2O$), sodium acetate trihydrate, and sodium carbonate were obtained from Vetec Química Fina Ltda (Duque de Caxias, RJ, Brazil). All reagents used in this work were of analytical grade. Seven bacterial strains namely *Bacillus cereus* (ATCC 11778), *Campylobacter jejuni* (ATCC 33560), *Clostridium perfringens* (ATCC 3624), *Escherichia coli* (ATCC 10536), *Listeria innocua* (ATCC 33090), *Staphylococcus aureus* (ATCC 23235), and *Salmonella* enterica serotype Typhimurium (ATCC 14028) were used. All microorganisms were from the National Institute for Quality Control and Health (INCQS), linked to the Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil. The Brain Heart Infusion Broth (BHI) and the Mueller Hinton (MH) broth were obtained from Merck (Burlington, Massachusetts, USA). *Artemia salina* was obtained from the company Artemia salina do RN (Natal, RN, Brazil).

Lemongrass essential oil extraction

The lemongrass leaves were harvested in a rural area located in the city of Serra Redonda, PB, Brazil (07º 10 '42 "S, 35º 40' 30"W). The essential oil was extracted according to the Brazilian Pharmacopeia (*13*) by steam distillation (SOLAB, model SL76/I). The distillation process was carried out for 3 hours, and the temperature was kept at 100 ºC. In brief, when the water boiled, the steam passed through the distillation flask, which contained the plant material (14.859 kg). Then, the essential oil was condensed as it passed through a cooled tube. After the distillation process, the extracted essential oil was dehydrated with anhydrous granular sodium sulfate (Na_2SO_4), collected in an amber glass vessel, and kept under refrigeration at 4 °C until further analysis. The yield was calculated from the oil mass ratio, measured using the density and the volume (mL) of oil extracted, and divided by the dry mass (g) of the sample (*14*), according to equation 1:

$$R(\%) = \frac{volume_{oil}.density}{mass}.100 \qquad /1/$$

Cytotoxic activity determined by the lethality test against Artemia salina (Brine shrimp)

LEO's toxicity was determined according to the methodology described by Meyer *et al*. (*15*). LEO at different concentrations (from 1 to 200 µg/mL) and solubilized in seawater and Tween 80 were used against *Artemia salina* larvae. Firstly, *Artemia salina* eggs were incubated in seawater under constant aeration at a controlled temperature (25 °C) for 48 hours to hatch. Then, ten *Artemia salina* larvae were added to the test tubes containing the LEO solutions and the control solution (without

LEO). Later, they were incubated in the presence of light for 24 hours. After that, the number of larvae survivors in each test tube was counted under a lighted background and the lethal concentration (LC_{50}) was calculated using the probit method by plotting the concentration of LEO responsible for the death of 50 % of the larvae population. Data were analyzed in triplicate.

Microencapsulation of lemongrass essential oil

The microencapsulation process was performed using two different formulations, one containing only AG and another containing a blend of AG/MD (3:1 *m/m*), according to the study of Souza *et al*. (*6)*. For each formulation, a solution (20 %) of the wall material incorporated with 25 % of LEO was used. AG and MD were dissolved in distilled water under stirring and maintained on hydration for 12 hours at 25 °C to ensure complete saturation of the polymer's molecules. Then, LEO was gradually added to the wall material solution and stirred at 3,000 rpm for 5 min using a homogenizer (Ultra-Turrax IKA T18, Wilmington, USA). The solution was sonicated (Branson Digital Sonifier®, Model 102C, Branson Ultrasonics Corporation, Danbury, USA) for 2 minutes at 240 W. The emulsion formed was used as the liquid source in the spray drying process. After the drying procedure, the powder was stored in metalized and hermetically sealed packages at 4 °C until further analysis. Data were analyzed in triplicate.

Chemical composition of free and microencapsulated lemongrass essential oils by GC-MS

The chemical composition of the free and microencapsulated LEO was analyzed by gas chromatography coupled with mass spectrometry (GC-MS), using a gas chromatograph Shimadzu TQ8040 (Shimadzu Corp., Kyoto, Japan), equipped with an RTx-5 capillary column of fused silica (30 m x 0.25 mm x 0.25 µm) in a similar procedure as described by Lu *et al*. (*16*). Firstly, the oil contained in the microparticles was pre-extracted with n-hexane solvent of spectroscopic grade (99.9 % purity, Sigma-Aldrich). For the separation procedure, the initial column temperature was at 60 °C for 1 min and then increased to 250 °C at a rate of 5 °C/min. Then, the EOs were diluted in n-hexane at a concentration of 150 ppm and 5 µL was injected into a GC system via split mode injection, with a ratio of 1:30, and an injection temperature of 250 °C and 57 kPa. Helium was used as a carrier gas at a flow rate of 0.99 mL/min. The mass spectrum was acquired with an ionization energy of 85 eV and a mass scan ranging from 40.0 to 450.0 m/z. The compounds present in the samples were identified based on the library database of the National Institute of Standards and Technology (NIST), in comparison with data available in the literature. Data were calculated according to the peak area of the chromatograms and expressed as a percentage.

Encapsulation efficiency

The encapsulation efficiency (EE) was determined by evaluating the amount of total phenolic compounds in the free and microencapsulated EOs, according to the methodology proposed by Cabral *et al*. (*9*) with modifications. The total phenolic compounds content analysis was performed on the methanolic extracts of the samples using the Folin-Ciocalteau colorimetric method as described by Singleton and Rossi (*17*). The extracts were obtained from 2 mL of the free LEO and 2 g of each microencapsulated LEO, both dissolved in 20 mL of methanol and gently stirred for one hour. After that, the solution was centrifuged at 5,000 rpm for 15 min, and an aliquot of 0.5 mL of the supernatant was collected for analysis. The absorbance was measured at 750 nm in an Epoch microplate spectrophotometer (Synergy-BIOTEK, USA) and the total microencapsulated phenolic compounds were shown in mg of gallic acid equivalents per g of sample (GAE mg/g). Data were analyzed in triplicate. EE was expressed as a percentage of phenolic compounds, according to equation 2:

$$EE = \frac{Total\ phenolic\ content\ of\ the\ microcapsule\ (\frac{mg}{100g})}{Total\ phenolic\ content\ of\ the\ oil\ (\frac{mg}{100g})}.100 \quad /2/$$

Microparticles morphology

The microparticles obtained from AG and AG/MD (3:1) were examined by Scanning Electron Microscopy (SEM) using a JEOL JSM 6360-LV microscope (Jeol Company, Tokyo, Japan). The samples were scattered on copper supports using double-sided adhesive tape and then covered with gold coating. The micrographs were obtained under vacuum and at 15 kV of voltage acceleration with magnification under 1,200x. The area of the granules was calculated using the ImageJ v. 1.51 software (*18*).

In vitro antioxidant potential by DPPH, FRAP, and ABTS assays

The antioxidant potential of the free and microencapsulated LEO was measured on the same methanolic extracts prepared in the “Encapsulation efficiency” section, and evaluated using different methods (DPPH, FRAP and ABTS). The DPPH free radical scavenging potential was determined according to the methodology of Brand-Williams, Cuvelier, & Berset (*19*). The analysis was performed in a microplate by adding 5 µL of the extract and 195 µL of a 125 µmol/L DPPH methanolic solution. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 517 nm using a spectrophotometer with a microplate reader.

The determination of the ferric iron reducing antioxidant power (FRAP) was performed by the method of Benzie & Strain (*20*). The assay was performed in a microplate by adding 10 µL of the sample and 300 µL of the FRAP reagent in each microwell. After 30 minutes of incubation at 37°C in

the dark, the absorbance was measured at 593 nm using a spectrophotometer with a microplate reader.

The antioxidant capacity to scavenge ABTS•+ radicals was performed using the method described by Re *et al*. (*21*), with some modifications. The ABTS•+ cation radical was prepared with 7 mmol/L of ABTS and 2.45 mmol/L of potassium persulfate solution (1:1, *V*/*V*). The working solution was stored for 12-16 hour at room temperature in the dark. Subsequently, the mixture was diluted with methanol, and the absorbance was adjusted to 0.700 ± 0.020 at 734 nm. Then, 300 µL of the ABTS solution and 3 µL of the sample were added to a microplate. The mixture was stirred and stored in the dark for 30 min. After incubation, the absorbance was measured at 734 nm using a spectrophotometer with a microplate reader.

The results were expressed in µmol of Trolox per 100g of LEO. The antioxidant analyzes were performed using external calibration curves, in which the gallic acid standard ranged from 0 to 1 mg/g. The calibration curves of Trolox ranged from 0.1 to 1.5 µmol/g (FRAP), 0.1 to 1 µmol/g (DPPH), and 0.038 to 2.4 µmol/g (ABTS); the detection limit were 1.22 mmol/L (FRAP), 1 mmol/L (DPPH), and 2.53 mmol/L (ABTS).

Antimicrobial activity determined by disk diffusion agar method

The microorganisms used in this study included bacterial strains selected due to their characteristics as pathogens. The antimicrobial effect of the free and microencapsulated LEO was determined according to the protocol proposed by the Clinical and Laboratory Standards Institute (CLSI) manual (*22*). Firstly, aliquots (100 µL) of each bacterial suspension at 10^8 CFU/mL previously cultivated in Brain Heart Infusion (BHI) broth at 37 °C for 24 hours were inoculated onto the surface of petri dishes containing Müeller-Hinton (MH) agar. Then, three paper discs with a diameter of 6 mm impregnated with 10 µL of free and microencapsulated 1 % (*V*/*V*) LEO in distilled water and tween 80 were evenly distributed on the plates. The plates were incubated at 37 ± 2 °C for 24 hours and, after incubation, the diameter of the growth-inhibition zones was measured using a digital caliper. The microorganisms' resistance to the free and microencapsulated LEO was analyzed according to the size of inhibition halos, which were classified into: resistant, for diameters less than 14 mm; intermediate, for diameters between 15 and 19 mm; and susceptible, for diameters greater than 20 mm (*22*).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) assay was performed according to the methodology described by CLSI (*22*). The bacterial suspensions (10^8 CFU/mL) were inoculated onto

the surface of petri dishes containing MH agar. Then, disks impregnated with the free and microencapsulated LEO at concentrations ranging from 1 % to 6 % (*V*/*V*) and a control (without LEO) were placed on the plates, which were incubated at 37 °C for 24 hours. After incubation, the disk with the lowest concentration of EO that inhibited the visible bacterial growth was considered the MIC value. The MIC value was analyzed in triplicate.

Statistical analysis

The data of three replicates were statistically investigated by one-way analysis of variance (ANOVA) using the SAS® version 9.0 software (SAS Institute Inc., Cary, USA) (*23*) licensed to the Federal University of Paraíba, and the significant differences between treatments were analyzed using the Sheffé's test when p-values were less than 0.05 ($p < 0.05$). Pearson's correlation was used to determine the correlation between the antioxidant methods and total phenolic compounds.

RESULTS AND DISCUSSION

Extraction yield

The yield of lemongrass essential oil was 0.45 %, corresponding to 67 mL of oil extracted by steam distillation, which is lower than the yields reported in the literature obtained by hydro-distillation extraction, which varied between 0.70 % and 0.95 % (*4,5*). This smaller yield obtained in this study may be related to the lemongrass variety, climate characteristics of the region where the lemongrass was produced, stage of plant development, as well as extraction conditions (*5*), factors that alter plant metabolism. It is important to emphasize that although the extracted LEO has a lower yield compared to the literature, its antioxidant and antimicrobial properties may justify future industrial applications.

Cytotoxic activity with Artemia salina

The *Artemia salina* toxicity test is a biological test considered one of the tools used for the preliminary toxicity assessment of bioactive compounds (*24*). The values of lethal concentration (LC) of lemongrass essential oil were: $5.31 < LC < 13.97$ (LC_{50}), $14.29 < LC < 90.25$ (CL_{90}), $17.81 < LC < 162.75$ (CL_{95}). There was a 100 % lethality for microcrustaceans from a concentration of 200 µg/mL. According to Meyer *et al*. (*15*), this test is an efficient bioassay that can be widely used by pharmacologists and chemists of natural products to detect and isolate plant constituents with biological properties. Moreover, it is practical, fast, safe, economical, and has a good correlation with cytotoxic activity in some human tumors, as well as anticancer, insecticide, molluscicide, and antifungal activities (25,26). In this sense, compounds considered toxic for *Artemia* cells may present some other biological properties such as antioxidants and antimicrobial.

The toxicity is considered low when $LC_{50} \geq 500$ µg/mL, intermediate when $LC_{50} \geq 100 \leq 500$ µg/mL, and toxic when $LC_{50} \leq 100$ µg/ mL (27). LEO showed a LC_{50} with a limit of $5.31 < LC < 13.97$ µg/mL. For lethal concentrations 90 and 95, the values ranged from 14.20 µg/mL to 162.75 µg/mL. LEO's LC_{50} toxicity can be attributed to the major compounds, mainly to the content of citral isomers, since these compounds show great biological properties including cytotoxicity, as already reported in other studies (28-30). Though the use of this essential oil in the food industry can benefit consumers; the amount applied should be carefully considered.

Morphology of the microparticles evaluated by Scanning Electron Microscopy (SEM)

The efficiency of a microencapsulation process can be indirectly evaluated by analyzing the morphology of the microparticle since structures with cracks or damages can compromise the carrying and the protection of the microencapsulated compound of interest (*8*).

Fig. 1 shows the microparticles prepared with pure AG and a blend of AG/MD (3:1, *m*/*m*). Images of the microparticles show that there was no evidence of cracks on the particles' surfaces, ensuring low gas permeability and better protection to the lemongrass essential oil. This is an important feature for microencapsulated essential oils as it ensures that their volatile compounds remain protected for a longer period, being released in a controlled manner when introduced into the food matrix.

Figure 1

A wrinkled structure was observed for both treatments, and it represents a characteristic of microparticles stabilized by the spray drying process (*6*). Therefore, differences in the wall materials formulation did not affect the morphological characteristics of the microparticles. As can be seen in Fig. 1a, the microparticles produced using only AG exhibited a higher presence of smooth granules than those using MD (Fig. 1b).

Chemical composition by GC-MS

Different compounds – *e.g.*, ketones, fatty aldehydes, alkenes, and terpenoids – were identified by analyzing the chemical composition of the free and microencapsulated essential oils. The components profiles (%) are exhibited in Table 1.

Table 1

The main compounds identified in the lemongrass essential oil, as well as in the microparticles were α-Citral (36.70 % for LEO, 36.28 % for AG, and 33.12 % for AG/MD) followed by β-Citral (22.92 % for LEO, 22.41 % for AG, and 21.80 % for AG/MD). Previous studies stated that the citral is the main compound responsible for the antimicrobial activity of LEO (*3,31*). Some components in low

concentrations were detected only in the non-microencapsulated essential oil, such as 3-Butenyl hexyl ether, α-Humulene, and γ-Cadinene. These compounds may have been volatilized during the heat treatment applied.

From the obtained results, it can be observed that the main components of interest (α-Citral and β-Citral) were retained after the drying process, with a significant percentage of the oil components. Although some amount of essential oil may be lost due to the high temperatures during the spray drying process, some of the main oil's components are still present in the microparticles containing LEO. Therefore, future industrial applications of LEO using AG or the mixture of AG/MD can be considered viable, since these encapsulation materials have shown to be efficient in retaining most of the compounds identified in the non-microencapsulated essential oil.

Encapsulation efficiency

The encapsulation efficiency (EE) of lemongrass essential oil (LEO) significantly differed ($p < 0.05$) between the encapsulating materials (Table 2). The AG microparticle presented an efficiency of about 88 % in the microencapsulation of lemongrass essential oil, while the mixture of AG/MD obtained 71 %. Similar results were found in the studies of Rajabi *et al*. (*32*), in which the same trend was observed during encapsulation of the bioactive components of saffron by spray drying using AG, MD, and gelatin as wall materials. Garcia *et al*. (*33*) also found a similar efficiency level (73.57 %) during the microencapsulation of basil essential oil using an AG-based formulation. In general, the spray drying process used to encapsulate essential oils can maintain the volatile components during the drying process (*6*).

Table 2

It was observed in this study that the addition of MD in the microparticles formulation led to a decrease in the encapsulation efficiency. This result may be related to the interaction between the bioactive compounds present in the essential oil and the wall materials used as encapsulants (*8*). Likewise, the matrix formed using AG/MD was not compact enough to retain a higher amount of LEO compared with the AG matrix. Furthermore, the temperature of the drying air used in the spray dryer can also influence the encapsulation efficiency (*8*).

Antioxidant capacity

The results of the antioxidant activity of lemongrass essential oil and the microparticles that contain it are described in Table 2. As expected, our results for antioxidant activity in ABTS, DPPH, and FRAP assays were higher for the non-microencapsulated lemongrass essential oil. Although the microparticles had shown lower antioxidant capacity compared to the free oil, the microencapsulation

process was able to maintain the bioactivity of the compounds. Consequently, the microencapsulation technology can keep the compounds stable during storage, maintaining their characteristic as an antioxidant material (*11,34*).

As described in Table 2, there was a significant difference ($p<0.05$) between the encapsulating materials used; the AG microparticles results were better in comparison to AG/MD microparticles. It was observed that microparticles containing microencapsulated LEO in AG showed an antioxidant capacity of 2042.30, 867.67, and 906.27 μmol TE/100g for ABTS, DPPH, and FRAP assays, respectively, compared to the microencapsulated LEO in AG/MD.

Comparing LEO with lemongrass essential oil from other species (*Cymbopogon citratus*), Fokom *et al*. (*2*) obtained lower values when evaluated the antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. These authors state that the antioxidant activity of the essential oil varies with the harvest periods and symbiotic status of the plant used to extract the essential oil, which can also cause variation between different species. The objective of microencapsulating the LEO is to reduce these variations suffered by it, due to the protection caused by the wall material that provides a low reduction when compared to free LEO. Da Silva *et al*. (*35*) showed that AG, as a wall material, showed better action in preserving bioactive compounds in spray dried products than other carriers. The same behavior was exhibited in this study. Possibly, this is due to the affinity of AG with the bioactive compounds present in the samples under study, leading to a thermoprotective effect while exposure to higher temperatures.

Antimicrobial activity

The antimicrobial activities of the free and microencapsulated LEO are shown in Table 3. According to the classification described by the CLSI (*22*), the strains of *C. perfringens*, *S.* Typhimurium, and *L. innocua* were resistant to the LEO microencapsulated in AG. While the Gram-negative bacteria *E. coli* and the *S. Typhimurium* were resistant to the LEO microencapsulated in AG/MD.

Table 3

B. cereus and *C. jejuni* were the most sensitive strains to the free LEO, presenting inhibition zones greater than 20 mm, and, therefore, were classified as susceptible (*24*). The citral contents can be associated with the high efficacy of LEO against these microorganisms. Yoplac *et al*. (*36*) studied the antimicrobial activity of citral and observed that the lowest concentration used (0.80 mg/mL) inhibited *B. cereus*. In another study, strains of *C. jejuni* were inhibited by a low concentration of LEO (0.018 %), and this activity was attributed to the citral content (*37*). Both authors reported that citral

can alter and penetrate the bacterial cell wall, leading to protein denaturation and cell membrane destruction, thus causing lysis and cell death.

LEO's inhibitory effect was reduced after microencapsulation, and it can be associated with the wall material of microcapsules. This material controls the release of the essential oil and its bioactive compounds due to the barrier the material creates. Thus, probably the exposure time of microorganisms to the microencapsulated EOs was not sufficient for their inhibition.

The strains of *L. innocua* and *C. perfringens* were not inhibited by the LEO microencapsulated in AG/MD, leading to the non-formation of an inhibition zone. De Souza *et al.* (*6*) studied the antimicrobial effect of orange essential oil microencapsulated in AG and MD. They reported that the addition of MD reduced the solubility of the microparticles. This lower solubility probably provided low antimicrobial activity because the LEO's mechanism of action on these microorganisms is through their membrane permeabilization (*38*). Thus, the passage of EO to the cell periplasm of the microorganisms was prevented. In addition, the AG/MD microparticles showed lower encapsulation efficiency (Table 2).

No studies evaluating the antimicrobial activity in microcapsules of LEO prepared with AG and MD as encapsulating materials were conducted. Leimann *et al.* (*3*) analyzed the essential oil of lemongrass (*Cymbopogon citratus*), which also presents citral as the main compound. In this study, they found that free and microencapsulated EO showed the same MIC for *E. coli* (22.32 mg/mL) and *S. aureus* (2.79 mg/mL), meaning that the encapsulation process did not cause deterioration of the essential oil.

The values for the Minimal Inhibitory Concentration (MIC) tests confirmed the results obtained with the agar diffusion method. The strains of *C. perfringens* and *L. innocua* did not present MIC values, and have not presented antimicrobial activity for LEO microencapsulated in AG/MD. On the other hand, *B. cereus* and *C. jejuni*, were inhibited at the lowest free LEO tested concentration (10.10 µg/mL). In addition, both microcapsules containing LEO also inhibited *B. cereus* at 10.10 µg/mL. These results confirm the high sensibility of the *B. cereus* and *C. jejuni* strains to LEO, as already reported in other studies (*36,37*).

Due to the greater inhibition zone for *E. coli*, the free LEO was more efficient when compared to the microencapsulated LEO. In relation to MIC, both microencapsulated oils presented a value of 30.92 µg/mL. Even though the microencapsulated LEOs have been less effective than the free oil, our results were better than those reported by Assis *et al*. (*39*), which studied the antimicrobial activity of free and microencapsulated LEO against *E. coli*. The authors obtained a MIC of 80 µL/mL. In another study, the antimicrobial activity of LEO and citral was evaluated against *E. coli* (*40*). The authors reported high inhibition of the microorganism at low concentrations with MIC values of 2.2

mg/mL and 1.0 mg/mL for LEO and citral, respectively. They also reported that this high activity is related to the mechanism of action of LEO and citral, which can degrade membrane proteins and increase the cell permeability of microorganisms.

The free LEO was more effective against *S. aureus*, with a MIC value of 10.10 µg/mL, than the microcapsules containing LEO, which presented MIC values between 20.41 and 30.92 µg/mL. The differences between the MIC values have been influenced by the microencapsulation process as well as the wall materials used. Overall, the microparticles produced with AG presented a better effect when compared to the microparticle using AG/MD (3:1) as wall materials.

CONCLUSIONS

The results obtained in this study show that microparticles containing lemongrass essential oil can be prepared, via the spray drying process, using Arabic Gum and Maltodextrin as wall materials, resulting in high microencapsulation efficiency, and that Arabic Gum is more effective in oil retention. In addition, the essential oil was not degraded by the spray drying process, demonstrating the gum's efficacy to encapsulate the oil and retain its compounds. The microparticles prepared using AG presented a more regular surface, higher antioxidant property, and better retention of citral compounds. The microparticles maintained antibacterial and antioxidant properties when compared to the free essential oil, which is of great interest to the food and pharmaceutical industries. In general, AG is considered a good encapsulating material for lemongrass essential oil for future elaboration of microparticles with industrial applications.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTION

Anely Maciel de Melo designed the study, collected experimental data, performed analyses, interpreted the results and wrote the manuscript. Rafaela Cristina Turola Barbi helped in the analysis of antioxidants and in the writing of the manuscript. Hugo Junior Barboza de Souza, Diego Alvarenga Botrel and Soraia Vilela Borges assisted and supervised the preparation of microcapsules, analysis of the characterization of the powders and writing of the manuscript. Atacy Maciel de Melo helped to write the manuscript. Max Rocha Quirino, Roberto Germano Costa and Solange de Sousa supervised the project and contributed to the writing of the article. All authors contributed to the interpretation of the data.

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Table 1. Major compounds of lemongrass essential oil (*Cymbopogon flexuosus*) and its microcapsules

Compounds	LEO		AG		AG/MD (3:1)	
	Area/%	t_r/min	Area/%	t_r/min	Area/%	t_r/min
2-Hexanone	1.11	3.069	1.11	3.061	1.61	3.057
3-methylcyclopentanone	0.67	3.962	0.40	3.950	0.63	3.945
Trans - 2,4 Hexadienal	0.06	4.530	0.04	4.516	0.07	4.512
1-Hexyn-3-ol	0.52	5.008	0.52	4.987	0.61	4.979
2-Pentene, 2,4-dimethyl	3.96	5.138	1.35	5.203	1.62	5.195
Hexane, 2-nitro-	5.78	5.573	1.87	6.111	2.34	6.100
α-Pinene	0.11	5.872	nd	nd	0.08	5.847
Camphene	0.68	6.300	0.47	6.283	0.65	6.274
3-Butenyl hexyl ether	1.44	6.400	nd	nd	nd	nd
3-Hexen-2-one	4.67	6.804	5.48	6.776	5.95	6.765
Myrcene	0.28	7.457	0.18	7.440	0.24	7.426
6-methyl, Hept-5-en-2-one	1.07	7.323	0.69	7.305	0.78	7.295
l-Limonene	0.93	8.790	0.59	8.767	0.74	8.756
Linalool	1.55	11.475	1.59	11.440	1.50	11.430
Citronellal	0.06	13.726	0.03	13.688	0.05	13.693
Isogeranial	0.35	14.234	0.29	14.199	0.31	14.195
β-Citral	22.92	17.642	22.41	17.608	21.80	17.585
α-Citral	36.70	18.983	36.28	18.950	33.12	18.921
Caryophyllene	2.57	25.543	0.31	25.500	0.38	25.496
α-Humulene	0.19	27.007	nd	nd	nd	nd
γ-Cadinene	0.48	29.921	nd	nd	nd	nd
Caryophyllene oxide	0.51	32.387	0.39	32.336	0.23	32.335
Other compouds	12.85	-	25.30	-	27.26	-
Total	99.46		99.29		99.97	

LEO: lemongrass essential oil; AG: microcapsule made with Arabic Gum; MD: microcapsule made with maltodextrin; Rt: retention time; nd: not detected

Table 2. Encapsulation efficiency and antioxidant activity of free and microencapsulated lemongrass essential oil

Samples	EE/%	ABTS TE/(μmol /100g)	DPPH TE/(μmol /100g)	FRAP TE/(μmol /100g)
LEO	-	(2352.89± 3.05)^{a}	(929.14 ± 0.21)^{a}	(1108.57 ± 0.23)^{a}
AG	(88.11 ± 2.99)^{a}	(2042.30 ± 0.05)^{b}	(867.67 ± 3.62)^{b}	(906.27 ± 0.33)^{b}
AG/MD (3:1)	(71.10 ± 0.17)^{b}	(1108.57± 0.33)^{c}	(348.66 ± 1.21)^{c}	(630.45 ± 0.97)^{c}

Data were analyzed in triplicate and expressed as mean ± s.d .Means followed by the same letter in the same column do not differ significantly ($p>0.05$) according to "Sheffè" test. LEO: lemongrass essential oil; AG: microcapsule made with Arabic Gum; MD: microcapsule made with maltodextrin; EE: encapsulation efficiency.

Table 3. Antibacterial activity of lemongrass essential oil and its microcapsules

Microorganisms	Treatments					
	LEO		AG		AG/MD (3:1)	
	ZI *d*(inhibition/mm)	MIC/ (μg/mL)	ZI *d*(inhibition/mm)	MIC/ (μg/mL)	ZI *d*(inhibition/mm)	MIC/ (μg/mL)
Gram-positive						
B. cereus	(27.33±0.58)^{a}	10.10	(14.50±1.50)^{b}	10.10	(12.00 ±1.00)^{b}	10.10
C. perfringens	(19.50±1.5)^{a}	20.41	(13.50±0.50)^{b}	30.92	n.d.	n.d.
L. innocua	(14.17±0.76)^{a}	30.92	(12.50±0.50)^{b}	10.10	n.d.	n.d.
S. aureus	(19.33 ±1.53)^{a}	10.10	(14.83±0.76)^{b}	20.41	(12.33±0.58)^{b}	30.92
Gram-negative						
C. jejuni	(22.83±0.29)^{a}	10.10	(18.50 ±0.50)^{c}	20.41	(20.00±1.41)^{b}	20.41
E. coli	(18.00±1.00)^{a}	20.41	(14.00±2.00)^{b}	30.92	(12.67±1.15)^{b}	30.92
S. Typhimurium	(14.75±0.75)^{a}	30.92	(13.66± 2.08)^{ab}	41.67	(10.50±0.50)^{c}	63.83

Data were analyzed in triplicate and expressed as mean ± s.d .Means followed by the same letter in the same line do not differ significantly ($p>0.05$) according to "Sheffè" test. LEO = lemongrass essential oil; AG = microcapsule made with Arabic Gum; MD= microcapsule made with maltodextrin. ZI = Zone of inhibition; MIC = minimal inhibitory concentration; n.d.= not detected.

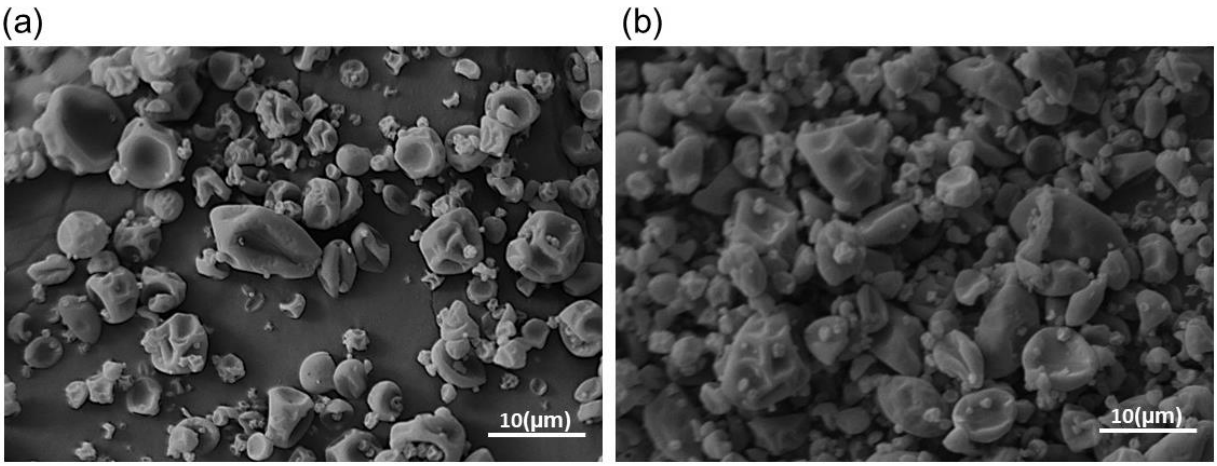

Fig. 1. Micrographs of microencapsulated lemon grass essential oil: a) AG and b) AG/MD