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Use of cashew gum combined with galactomannan for encapsulation of *Rosmarinus officinalis* essential oil

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ABSTRACT

Encapsulating matrices are important to maintain the properties, promote the low and controlled release, and avoid these bioactive compounds' undesirable reactions. This study's objective was to evaluate cashew gum (CG) use combined with galactomannan (GAL) in the rosemary essential oil encapsulation by atomization. During the emulsification, the polysaccharides were crosslinked with sodium trimetaphosphate. The particles obtained after encapsulation were evaluated for moisture, solubility, particle size, encapsulation efficiency, morphology, antimicrobial activity, and chemical composition of the essential oil volatiles. GAL/CG blend showed higher encapsulation efficiency and lower oil release than the cashew gum matrix. Undoubtedly, galactomannan combined with cashew gum improved the microparticles' characteristics due to the galactomannan's high emulsifying property even in low concentration. Essential oil maintained its antimicrobial and chemical properties practically after the encapsulation procedure.

Keywords: Natural polysaccharides, atomization, microencapsulation, essential oil.

Introduction

Blends combine characteristics of individual polymers that contribute to increased encapsulation efficiency and protection of the nucleus material. Polysaccharides obtained from exudates and plant seeds have been widely studied as encapsulation matrices (Souza et al., 2015; Botrel et al., 2017), and their combination may result in new encapsulants of bioactive substances, including those of a hydrophobic nature.

Cashew gum (CG) is an *Anacardium occidentale* exudate, being easy to isolate and obtain in the cashew producing regions. It consists of a major β -D-galactose chain (1 \rightarrow 3) associated with galactose and glucose lateral chains (Pitombeira et al., 2015), presenting approximately 6% polysaccharide-protein complexes (Paula & Rodrigues, 1995). The gum

possesses emulsifying properties and a low viscosity with an activation energy of flow for a solution at 2 and 3% (6 kJ mol^{-1}), characteristic of systems with little intra and intermolecular interactions (Kumar et al., 2012). Although cashew gum presents emulsifying properties (Porto & Cristianini, 2014), polysaccharides blends could improve essential oils' encapsulation efficiency (Oliveira et al., 2014; Fernandes et al., 2016).

Galactomannans (GAL) are neutral polysaccharides composed of a linear chain of mannose residues bound by β (1 \rightarrow 4) glycosidic bonds, to which galactose residues are bound through α -bonds (1 \rightarrow 6). GAL is easily found in legume seed endosperm and have thickening and stabilizing properties. They are non-toxic hydrocolloids, non-absorbable in the gastrointestinal tract, and non-soluble dietary fiber

(Cerqueira et al., 2011; Jiang et al., 2011; Buriti et al., 2014). They are also very viscous polysaccharides in aqueous solution and could improve emulsion characteristics prepared from cashew gum even in low concentration.

In particle formation by encapsulation, natural polysaccharides can be modified with chemical substances to alter their characteristics, aiming for gains at the end of the process. Chemical crosslinking displays various changes in the gum structure, originating a new material with characteristics that are many times different from those of the source material. This process can present several benefits to encapsulation: a more rigid particle-matrix wall improved mechanical and thermal resistance, and better retention and protection of the bioactive compound (Ribeiro et al., 2015). Sodium trimetaphosphate (TMFS) is a low-cost salt that is already employed in the food industry and is considered an effective crosslinking agent for several gums (Muhammad et al., 2000; Ribeiro et al., 2015).

Rosmarinus officinalis L. (rosemary) essential oils are flavoring, and their antioxidant activity justifies their wide range of applications, including food preservation (Hamre et al., 2010), cosmetics composition (Lee et al., 2011), and the formulation of nutraceutical and phytomedicine products (Ibarra et al., 2010). Rosemary is a very frequently used herb in the food industry, and its extracts are added to some products to improve oxidative stability and organoleptic properties (Sui et al., 2012). However, like other oils, rosemary essential oil is sensitive to external factors such as heat, light, and oxygen (Huynh et al., 2008). Therefore, microencapsulation is an alternative to provide better protection and physical-chemical stability for rosemary essential oil. Spray drying is an encapsulation technique entirely used by industry for bioactive encapsulation. In lipophilic compounds, they can be dispersed in oil-in-water emulsions and later encapsulated by a spray dryer (Christensen et al., 2001; Hernandez-Sanchez et al., 2015; Boger et al., 2018; Tomazelli Junior et al., 2018).

In this study, GAL/CG blends were evaluated as an encapsulant matrix of rosemary essential oil by a spray dryer, and the physicochemical and morphological properties of the produced particles were compared to CG matrix. In the final, the encapsulation process's influence was evaluated for the essential oil's chemical and biological properties.

Material and Methods

Materials

The rosemary (*Rosmarinus officinalis*) essential oil was purchased from Ferquima Ind. And Com. Ltd. The cashew gum was obtained from the Embrapa Tropical Agroindustry Experimental Campus. The *Caesalpinia pulcherrima* (galactomannan) seeds were handpicked in Fortaleza's metropolitan area and identified in the Prisco Bezerra Herbarium (excerpt numbers 563667 - *Caesalpinia pulcherrima*). All chemical reagents used were of analytical grade.

Cashew gum isolation

Crude gum was taken to the laboratory and isolated according to the methodology described by Torquato et al. (2004) with some modifications. The gum was ground in a mill and dissolved in water in a ratio of 1:4 (w/v) under stirring for 4 hours (without heating). Centrifugation was performed at 10,000 rpm for 10 minutes, and the precipitate was discarded. Filtration and precipitation with commercial ethanol (96°GL) were subsequently performed with an ethanol to gum ratio of 3:1 (w/w) under refrigeration for 24 hours. The precipitate was isolated and put to dry at 60°C. Finally, the gum was triturated and passed through a 212 µm-pore sieve. The polysaccharide was kept in a hermetically sealed glass for later use.

Galactomannan extraction from *Caesalpinia pulcherrima* seed endosperm

Caesalpinia pulcherrima seeds were ground in a mill and separated into teguments, cotyledons and endosperms. The endosperms were heated at 70°C in ethanol, refluxed for 15 minutes, and left to swell at the proportion of 1:10 (endosperm to distilled water) for 24 hours at 7°C. The intumescent endosperms were homogenized in mills with distilled water until a viscous solution was obtained. The material was filtered through a nylon mesh, and the filtered solution was precipitated with 96% ethanol in the ratio of 1:2 (v/v) solution/ethanol. The residue was submitted to the alcohol extraction/precipitation process. Finally, the precipitate (galactomannan) was dehydrated with acetone, dried in a forced-air circulation oven, and kept as a dry powder for later use (Cerqueira et al., 2009).

Encapsulation of the essential oil

This assay consisted of 2-step emulsification. At first, direct emulsification was prepared with wall material and oil. At this moment was realized the crosslinking of polysaccharides. Second, emulsifiers, Tween 80 and Span 80 were mixed to the first emulsion. In

preparing the first emulsion, polysaccharides were initially dissolved in distilled water and maintained under magnetic stirring for 12 hours at room temperature. The solution was then homogenized using an Ultra-Turrax (IKA, T25 digital) at 20,000 rpm for 1 min and scattered on ultrasound at 40x magnitude and 1 cycle per 30 seconds. After that, 5% (w/v) rosemary essential oil and 6% (w/v) crosslinking agent (TMFS) were added and stirred for 30 min at pH 12 (2M NaOH

was used for the pH adjustment). After this process, the solution was neutralized to pH 7 (2M HCL was used for pH adjustment). Next, the emulsifiers Tween 80 and Span 80 were added to the first emulsion 0.25 g and 0.75 g, respectively, and homogenized in the Ultra-Turrax (IKA, T25 digital) at 14,000 rpm for 5min. Finally, liquid emulsions (Table 1) were submitted to a spray-dryer (Model MSD 1.0, Labmaq do Brasil, Ribeirão Preto, Brazil).

Table 1. Description of each treatment used in the atomization process. GAL/Cross-linked CG = galactomannan and cross-linked cashew gum; Cross-linked CG = cross-linked cashew gum; TMFS = Sodium trimetaphosphate. Font: Mendes et al. (2020).

Treatments	Wall Material (g.100 mL ⁻¹ of solution)			Nucleus (g.100 mL ⁻¹ of solution)
	Cashew Gum	Galactomannan	TMFS	Rosemary Oil
Cross-linked GAL/ CG	19.4	0.4	6	5
Cross-linked CG	19.8	---	6	5

Microparticle characterization

Moisture content

The encapsulated material moisture content was determined through the Association of Official Analytical Chemists (AOAC, 2007). The moisture content was calculated in percentage (%) based on the weight loss after oven drying at 105°C until a constant weight was achieved.

Hygroscopicity

Hygroscopicity was determined based on the method proposed by Cai and Corke (2000), with some modifications. The powder samples from each treatment (approximately 1 g) were placed in a desiccator containing a saturated NaCl solution (75% relative humidity) at 25°C. The samples were weighed after one week, and the hygroscopicity was expressed as grams of moisture adsorbed per 100 g of dry solids (g.100 g⁻¹).

Solubility in water

Sample solubility was determined according to the modified methodology described by Cano-Chauca et al. (2005). For the analysis, 0.25 g of the sample was added to 25 mL of distilled water. The solution was centrifuged at 3000 rpm for five minutes, and its precipitate was discarded. Then, 20 mL of the supernatant was dried in an air circulation oven at 105°C for five hours. Solubility was calculated by the difference between final and initial mass, and the results expressed as solubility percentage.

Particle size distribution

Particle size distribution was analyzed using a Malvern 3000 Zetasizer NanoZS laser light scattering instrument (Malverne Instruments, UK). The microparticles were suspended in 25 mL of isopropyl alcohol (refractive index:1.39) at the ratio of 1:100 g mL⁻¹. The volume amount of 1 mL was used for particle size distribution analysis, and the average diameter was determined considering the diameter of a sphere of the same volume - De Brouckere Diameter D [4,3] and the average Sauter diameter - D [3,2].

Particle morphology

Particle morphology was evaluated by scanning electron microscopy (SEM). The particles were adhered to double-sided adhesive tape and mounted on microscope stubs with a 1 cm diameter and 1 cm height. The samples were then covered with a thin layer of gold through sputtering and examined by scanning electron microscopy (SEM Zeiss DSM, Model 940), which was operated at 15 kV with an image magnitude of 6700 X.

Encapsulation efficiency

Surface oil content was determined using petroleum ether as an extractor (García et al., 2006; Jafari et al., 2008). The spray-dried powder (10 g) was dispersed in 25 mL of solvent and stirred for 10 minutes at room temperature. The dispersion was filtered, and the particles washed with 5 mL of petroleum ether three times. After, the organic solvent was evaporated at room temperature by the gas exhaust fume hood. Surface oil mass was assessed and expressed about the initial mass of the particles used.

Total oil content was determined by a Clevenger apparatus for 3 hours, using 10 g of powder encapsulated in 250 mL of distilled water and multiplied by its density (0.915 g.mL⁻¹).

Encapsulation efficiency (EE) was calculated using the following Equation 1:

$$EE\% = [(total\ oil - surface\ oil)/total\ oil] \times 100 \quad Eq.(1)$$

Release of rosemary essential oil

Essential oil release was measured under accelerated conditions by hydrodistillation of the microcapsules in a Clevenger type apparatus. The microcapsules were vacuum filtered and washed three times with petroleum ether to remove non-microencapsulated essential oil. Essential oil extraction was carried out for 180 minutes, and the rosemary essential oil extraction volume was determined every 20 minutes.

Determination of minimum inhibitory concentration (MIC)

The MIC of the *Rosmarinus officinalis* essential oil on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar *Choleraesuis* (ATCC® 10708™) e *Pseudomonas aeruginosa* was performed through the microdilution method in Brain Heart Infusion (BHI) culture broth, according to the methodology described in CLSI (2017). Each 96 well round-bottomed, sterile, well-capped microplate was designed for analysis of a microorganism. Chlorhexidine was used as a positive control. Each of the microdilution plate wells received 100 µL of BHI broth, 20 µL of essential oil emulsion in different concentrations, and 80 µL of the microbial suspension. The microplates were incubated for 24 hours in a bacteriological oven at 37°C. Visual inspection of microbial growth was then performed after this period. MIC was considered the lowest concentration of essential oil, capable of inhibiting the tested strains' growth, as evidenced by the absence of visible microbial growth's visible turbidity.

Chemical analysis of the encapsulated oil

Pure rosemary essential oil extracted from the microparticles was diluted in hexane PA (10 µL: 990 µL) and analyzed through a CG-EM

Agilent gas chromatography with mass spectrometry, Model CG-7890B/MSD-5977A (quadrupole). The equipment conditions were as follows: electron impact at 70 eV, HP-5MS methylpolysiloxane column (30 m x 0.25 mm x 0.25 µm, Agilent), helium carrier gas with flow 1 mL min⁻¹ (8.8 psi) and constant linear velocity of 36.8 cm s⁻¹, injector temperature of 250°C, detector temperature of 150°C. Gas chromatography oven programming: initial temperature of 70°C, with a heating ramp of 4°C.min⁻¹ to 180°C and an addition of 10 °C.min⁻¹ to 250°C at the end of the run (34.5 min). Compound identification was performed by comparing the fragmentation patterns displayed in the mass spectra with those present in the database provided by the equipment (NIST version 2.0 of 2012 - 243,893 compounds) and literature data.

Statistical analysis

Variance analysis was performed using Statistica 10 software (Stat Soft, Inc., Tulsa, USA). The comparisons between the averages from three repetitions were performed using Student's T-test at 5% probability level ($p \leq 0.05$).

Results and Discussion

The GAL combined with CG confers a suitable viscosity of the aqueous phase at high solids concentration, and this fact favors a lot the encapsulation by a spray dryer. Moreover, the polysaccharides chosen are not reactive. Thus, they do not react with the encapsulated component during the process and upon storage, providing protection against environmental factors and leading to powders with the right end-use properties.

Particle characterization

The study of particle reconstitution properties is necessary for food products to understand their behavior during the processing, transformation, commercialization, and consumption of the final product (Fernandes et al., 2016). These properties are influenced by particle size and morphology, in addition to the presence of oil on the microparticle surface (Dima et al., 2016). Some characteristics of the formed particles are shown in Table 2.

Table 2. Moisture, hygroscopicity, solubility, particle size distribution, and encapsulation efficiency of galactomannan/cashew gum (GAL/CG) and CG microparticles. Font: Mendes et al. (2020).

Treatments	Moisture	Hygroscopicity (%)	Solubility	Particle size distribution		Encapsulation efficiency (%)
				D _{4.3} (µm)	D _{3.2} (µm)	
Crosslinked	1.97 ^a ±0.11	13.16 ^a ±0.29	72.25 ^a ±1.35	4.84 ^a ±0.82	4.68 ^a ±0.86	83.80 ^a ±3.63

GAL/ CG						
Cross-linked CG	2.12 ^a ±0.45	12.82 ^a ±0.69	70.92 ^a ±1.12	1.71 ^b ±0.31	1.66 ^b ±0.30	76.16 ^b ±1.74

Different letters indicate a significant difference between the samples ($p \leq 0.05$).

Moisture content plays a significant role in establishing the shelf-life of dry powders (REF). Therefore, higher moisture content facilitates microbial growth and agglomeration, which may affect physical and chemical stability and overall acceptability (Goyal et al., 2015). The particles did not display a significant difference ($p > 0.05$) between moisture treatments, presenting 1.86% content for GAL/CG and 2.57% for CG (Table 2). In general, moisture content of 3-4% is the minimum specification for most dry powders used in the food industry (Goyal et al., 2015); consequently, the values found were considered low and within a range that guarantees a long shelf-life. The powders' moisture content produced by spray-drying is strongly influenced by air humidity and the drying process temperature (Finney et al., 2002), variables that were kept constant (Finney et al., 2002). Similar results involving other essential oils were reported by (Botrel et al., 2012; Fernandes et al., 2012).

Water absorption is a critical factor in microencapsulated aroma's shelf-life because water can influence lipids' oxidation and the loss of flavoring compounds. The quality of preserved foods depends on the moisture content, migration, and absorption of the food during storage (Fernandes et al., 2014). There was no significant difference between the two samples ($p > 0.05$) in particle hygroscopicity, ranging from 12.13% and 13.45% (Table 2). Regarding relative humidity, 75% of the particles presented no agglomeration or visible change in powder structure. However, continuous moisture adsorption may result in powder caking through liquid bridges formation between powder particles (Botrel et al., 2014). Similar values of 9.3% to 13.9% were found in rosemary essential oil microencapsulation by spray drying with maltodextrin and modified starch as wall materials (Barros-Fernandes et al., 2014).

To be useful, the powders used as ingredients in the food industry must also present good solubility (Fernandes & Vilela, 2014). Solubility is the last stage of particle dissolution and is considered a decisive factor for analysis, such as product quality in powders (Jayasundera et al., 2011). Pure rosemary essential oil does not solubilize in the water at room temperature; however, it is possible to use particles in an aqueous medium due to the microencapsulation process. All particles were reasonably soluble,

ranging from 69.8% to 73.6% (Table 2), despite the core material's hydrophobic nature.

In literature, a solubility of 60% for the spray drying process is reported, in some cases reaching values of up to 90%, depending on the interfacial biopolymer material used (Cano-chauca et al., 2005). This variable is generally highly influenced by the carrier type (Yousefi et al., 2011) and the consequent presence of hydrophilic sites in the matrix structure. The galactomannan and cashew gum blend presented statistically equal solubility ($p > 0.05$) and structures that facilitate water interaction. The mannose/galactose ratio influences the galactomannan solubility in the polysaccharide chemical structure. In general, the higher the D-galactose content, the greater its solubility in water; however, *Caesalpinia pulcherrima* galactomannan has a ratio of 2:1. Although galactose content in galactomannan chemical structure is small, the amount of this polysaccharide used to form the microparticles was insufficient to influence particle solubility.

The particle size of the microencapsulated oils is another important factor since it is associated with oil stability and retention in the particles (Tontul & Topuz, 2012). It was observed that particle size was significantly influenced by the type of wall material ($p \leq 0.05$), with the highest values found when the galactomannan and cashew gum blend was used. This larger size of the particles formed by the blend can be explained by the higher emulsion viscosity produced in this treatment (data not showed). Emulsions with greater viscosity display higher resistance to flow, and the droplets formed during atomization will be more massive (Janiszewska & Witrowa-Rajchert, 2009). Galactomannan has a high viscosity characteristic even at low solids concentrations, being an interesting and efficient thickening agent for food.

One of the most important quality parameters for essential oil encapsulation is microencapsulation efficiency. Efficiency values ranged from 74.42 to 87.43%, and there was a significant difference ($p \leq 0.05$) between the two treatments (Table 2). The galactomannan and cashew gum blend wall material presented an encapsulation efficiency of 83.80%, higher than that of the cashew gum, which was 76.16%. This difference in encapsulation efficiency between treatments may be associated with polysaccharides' chemical structure and their

molar mass, especially galactomannan. This polysaccharide has a linear chain of mannose residues linked by β glycosidic bonds (1 \rightarrow 4), to which galactose residues are bonded by α -type bonds (1 \rightarrow 6). It has a molar mass of 2.48×10^6 g.mol⁻¹, much larger than that of cashew gum, and with a branched chemical structure consisting of a β -D-galactose (1 \rightarrow 3) major chain associated with galactose and lateral glucose chains with a molar mass of 1.44×10^5 g.mol⁻¹. Therefore, galactomannan incorporation into the blend as a wall material provided better protection of the

essential oil due to its thickening and stabilizing characteristics.

Particle morphology

The microparticle morphology of rosemary essential oil is shown in Figure 1. Concerning external morphology, most of the microparticles displayed a spherical shape with irregular concavities similar to craters and hollows, known as a “deflated ball” effect; particle size was also sharply diversified, characteristics common in spray-dried particles.

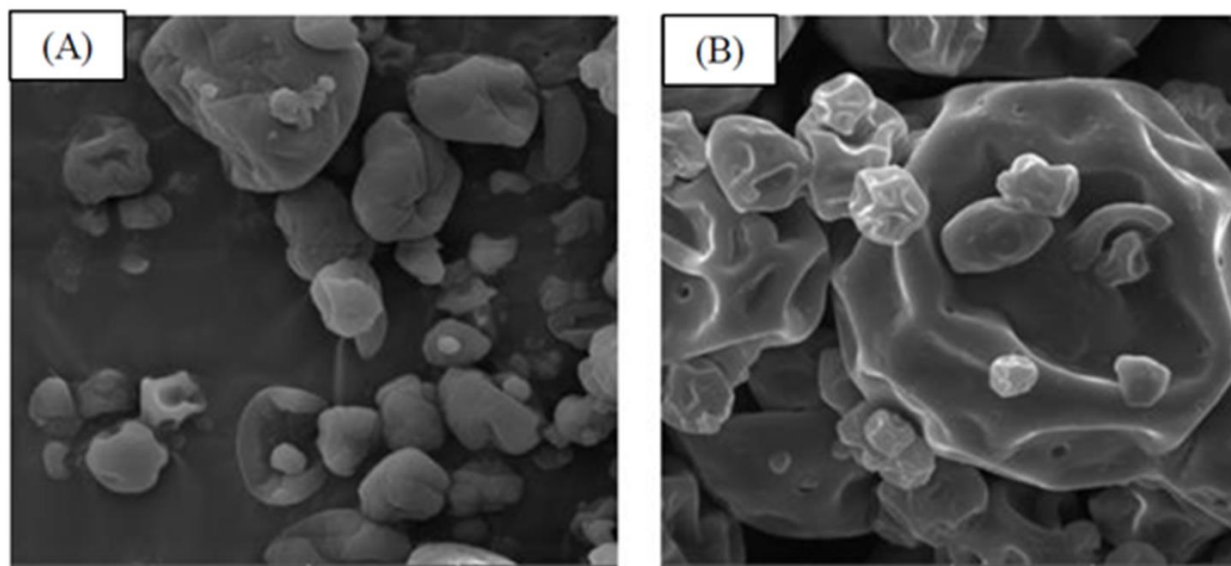


Figure 1. Scanning electron micrographs obtained for rosemary essential oil microparticles produced with the wall materials: (A) GAL/CG and (B) CG. Font: Mendes et al. (2020).

According to Ré (1998), surface depressions are formed due to the particles shrinking during drying and cooling. There was no evidence of fissures, porosity, cracking, or breakage on the particles produced from the two matrix types, which is an important feature to ensure low permeability to volatile compounds and better protection against rosemary essential oil oxidation (Botrel et al., 2012). Observation of the external microparticle microstructures verified that the galactomannan and cashew gum blend presented a higher proportion of spherical particles and less roughness. It is probably since the galactomannan provides elasticity to the particle during the drying process as a matrix (Fernandes et al., 2016).

Chemical composition of rosemary essential oil

Figure 2 represents the main components found in pure rosemary essential oil and the oil retrieved from the capsules. The compositions of pure rosemary oil and encapsulated rosemary oil in the GAL/CG and CG were remarkably similar,

indicating the preservation of these compounds and probably the oil characteristics after the process. Only minor differences in the percentage of components were observed. The aromatized compounds' characteristics should always be evaluated to verify possible changes in oil composition due to the encapsulation process and the impact of these changes on the final product. Component 1,8-Cineol present in the oil recovered from the microparticles was higher than in pure oil, possibly due to its concentration concerning the other volatilized components, although in a small amount.

In contrast, the percentage level of α -pinene in pure oil was higher than that found in the oil recovered from the microparticles, indicating a more significant loss of this component during the heating process in particle formation. In encapsulation, the volatile hydrophobic compounds present on the particles' surface are less protected and more sensitive to evaporation (Baranauskiené et al., 2006). A study by Fernandes & Vilela (2014) analyzed the

composition profile of rosemary essential oil after spray drying encapsulation with different encapsulation matrices, in which an expressive reduction in the 1,8-Cineol and β -pinene components was observed. Some rosemary essential oil components such as α -pinene, 1,8-

cineole, and camphor possess proven antimicrobial and antioxidant activities; therefore, their maintenance in microencapsulated oil is essential (Baranauskiené et al., 2006; Santoyo et al., 2005).

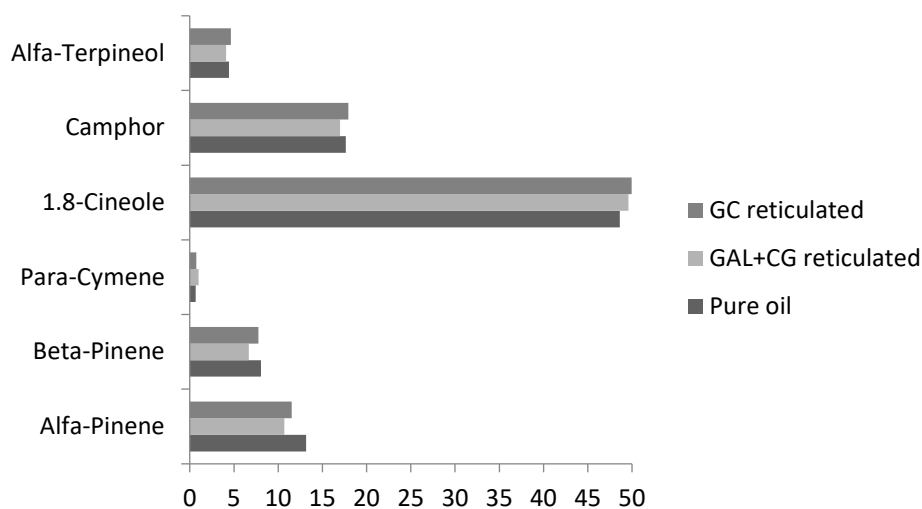


Figure 2. Percentage profile of the major components analyzed by gas chromatographic-mass spectrometry (GC-MS) of pure rosemary essential oil and from GC/GAL and CG microparticles. Font: Mendes et al. (2020).

Rosemary essential oil release

In general, the encapsulated ingredient's release depends on the particle geometry and the encapsulating agent employed. These factors dictate the release mechanism that may be based on solvent effects, diffusion, degradation, or fracture. Some stimuli can accelerate the release rate of the encapsulated ingredient, such as pH change, mechanical stress, temperature, enzyme activity, time, and osmotic strength, among others (Baranauskiené et al., 2006; Desai & Park, 2005).

Literature has recently reported the quantification of essential oil release through gas chromatography, spectrophotometry, and thermogravimetric analysis (Chang et al. 2006; Maji & Hussain, 2009; Beirão-da-Costa et al., 2013). However, the hydrodistillation technique was used in this work to evaluate oil release under accelerated conditions by heating the microparticles (Figure 3). The CG microparticles displayed a maximum oil release point at 78 minutes, which was calculated from the parabola vertex relative to the quadratic function indicated in Figure 3. Therefore, the swelling of the polymeric matrix takes place in the first 78

minutes with the determination of the “burst effect”, associated with rapid solubilization in the release medium of the active agent on the microparticle surface (Bazzo et al., 2008). After this time, it began to considerably decrease, presenting a release near zero in 180 minutes, meaning complete relaxation and swelling of the polymer matrix and great diffusion of the active agent present in the microparticles (Bazzo et al., 2008).

In EO release from GAL/CG microparticles, the maximum oil release point was at 128 minutes. After this period, the release was diminished, and 0.42 mL of rosemary essential oil was recovered after 180 minutes. The microparticles formed by the blend provided better oil release modulation. The presence of galactomannan with its more extensive and more packed molecular structure must have contributed to this oil release characteristic.

In this manner, it is possible to attest that the GAL/CG microparticles present better essential oil retention over time in various heating conditions than CG.

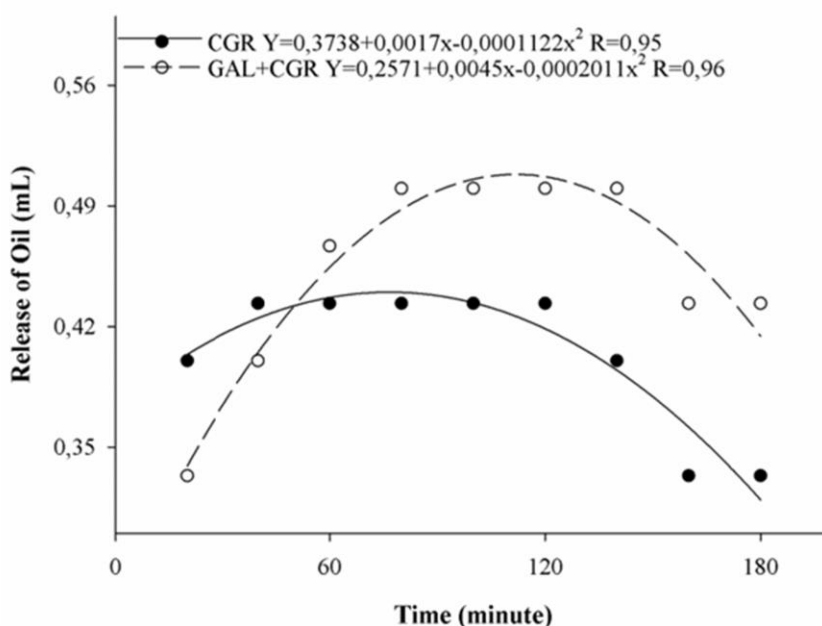


Figure 3. Rosemary essential oil release from CG/GAL and CG microparticles submitted to accelerated condition. Font: Mendes et al. (2020).

Determination of minimum inhibitory concentration (MIC)

The encapsulated essential oil was recovered from the microparticles through hydrodistillation and had its antimicrobial activity

compared to a pure essential oil (before encapsulation). The percentages of the compounds identified were shown in Figure 2. Table 3 shows the minimum inhibitory concentration values for pure essential oil and encapsulated essential oil.

Table 3. Minimum inhibitory concentration (MIC) of pure rosemary essential oil and the oil extracted from microparticles for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* e *Salmonella enterica* subsp. *enterica* serovar Choleraesuis (ATCC® 10708™). Font: Mendes et al. (2020).

Samples	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. Coli</i>	<i>S. enterica</i>
	mg mL ⁻¹			
Pure oil	10	10	5	10
After encapsulated by Cross-linked GAL/CG	10	10	10	10
After encapsulated by Cross-linked CG	10	10	10	10

The α -pinene compound percentage underwent a small reduction during the encapsulation process, which may have contributed to the increase of the minimum inhibitory concentration for the *E. coli* strain after encapsulation from 5 mg.mL⁻¹ to 10 mg.mL⁻¹. On the other hand, the minimum inhibitory concentration was the same for other tested microorganisms. This result indicates that the essential oil maintained its biological activity after all the encapsulation process procedures. Other studies in literature also reported the non-influence of the encapsulation method on essential oils' antimicrobial activity (Fernandes et al., 2008; Leimann et al., 2009).

Some authors affirm that rosemary essential oil's antimicrobial activity is related to its volatile components such as 1,8-cineol, camphor, eugenol, and α -pinene (Ojeda-Sana et al., 2013;

Teixeira et al., 2013). It is quite difficult to attribute the antimicrobial effect to only one active principle because the essential oil contains a mixture of different chemical compounds, and its antimicrobial activity may result from a synergistic effect of its components (Jiang et al., 2011).

Conclusion

Encapsulation of essential oil is especially useful to retain its chemical and biological properties for a longer time, given the substance's volatility. Low-cost and non-toxic matrices such as natural polysaccharides can be used to protect and integrity essential oil. In this work, we showed the positive effect of low concentrations of galactomannan to cashew gum emulsion, which resulted in the improvement of the emulsifying properties and, consequently, higher efficiency of

encapsulation and lower liberation of the oil from the microcapsules. The procedure adopted to increase the emulsification property and resistance of the wall material of the microparticles, associated with the encapsulation method chosen, not impaired the biological and chemical properties of the essential oil.

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