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Microencapsulation of Essential Oil from *Campomanesia adamantium* Residue with Antioxidant Capacity Retention

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Abstract

The essential oil (EO) extracted from the peels and seeds of guavira (*Campomanesia adamantium*) was microencapsulated via complex coacervation between gelatin and gum arabic, followed by freeze-drying. This process aimed to reduce the volatility of the essential oil and protect bioactive compounds through the microcapsule wall. For process optimization, the influence of proportions of wall materials (gelatin: gum arabic ratios of 1:1, 1:2, 1:3, 2:1, and 3:1) and EO quantity (20% - 42.8%) on antioxidant capacity, morphology, and microencapsulation yield was measured using a central composite rotational design (CCRD). Additionally, the chemical composition and EO retention rate in the microcapsules were assessed. Gelatin: gum arabic ratio of 1:2 and EO quantity of 40.3% resulted in superior results, with the highest antioxidant capacity, a microencapsulation yield of 68%, and spherical morphology. Notably, the incorporation of a higher amount of EO led to an increase in the antioxidant capacity, with values reaching up to 99% equivalent to pure oil. All formulations maintained the same pure EO's main constituents, including α-pinene, limonene, β-ocimene, and β-caryophyllene, indicated by gas chromatography coupled to mass spectrometry. Consequently, for the first time, EO microcapsules were successfully obtained from guavira residue, showing high microencapsulation yield and EO retention. This achievement adds sustainable value to residue normally discarded, which enables better use of residue generated by the food industry. Due to the preservation of its antioxidant capacity and enhanced retention of volatile compounds, these microcapsules promise applications in the food, pharmaceutical, and cosmetic industries, aligning with sustainability principles.

Keywords: antioxidants, Cerrado, microcapsule, plant oils, sustainability, volatile oil

Abbreviations: antioxidant capacity (AC); central composite rotational design (CCRD); essential oil (EO); ferric reducing antioxidant power (FRAP); gelatin (G); gum arabic (GA); microcapsule (MC); microencapsulation essential oil (MEO); microencapsulation yield (MY); retention rate (RR); 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ); wall material (W).

Highlights

First microencapsulation study of essential oil (EO) from guavira fruit residues.

Encapsulated EO from guavira residue has an antioxidant capacity similar to pure EO.

Complex coacervation/freeze-drying efficiently retained the major compounds of EO.

Study variables led to powdered microcapsules with potential application.

1. Introduction

Sustainability is now more vital than ever as we confront global environmental issues like climate change and resource depletion (Abbass et al., 2022). One standout eco-friendly and economically sound practice is the efficient utilization of fruit residue, contributing to environmental preservation and sustainability (Alves et al., 2020). An excellent example of this sustainable usage is extracting active ingredients from fruit peels and seeds, for applications in cosmetics, pharmaceuticals, and food (Viscardi et al., 2017; Suleria et al., 2020). This helps to value the residue and also opens doors for new businesses and revenue streams. For instance, essential oils (EO) with bioactive properties can be extracted from these residues.

Essential oils or volatile oils are aromatic liquids obtained from various plant portions and are chemically constituted by terpenes and their derivatives (Viscardi et al., 2017; Sá et al., 2018). EO have been used in food (Bonilla et al., 2018; Eghbal & Choudhary, 2018), perfumery, cosmetic (Gnatta et al., 2021), and pharmaceutical (Selles et al., 2020) areas, since they already have numerous recognized biological activities, such as anti-inflammatory (Nascimento et al., 2018), analgesic (Viscardi et al., 2017), antitumor (Nascimento et al., 2018), antioxidant (Ferreira et al., 2021; Valarezo et al., 2021, 2022), antibacterial (Sundararajan et al., 2018), and antifungal (Silva et al., 2022). In addition, EO stand out for its aromatic properties (Radünz et al., 2019), however it is volatile and susceptible to oxidation and degradation in the presence of factors such as light, oxygen, heat and moisture (Bastos et al., 2020), which may reduce biological potential. EO stability and maintenance of bioactive components can be obtained by microencapsulation (Khatibi et al., 2021).

Among the microencapsulation methods, complex coacervation is a technique appropriate to hydrophobic compounds as core materials. The technique is quite versatile and can provide protection against degradative reactions, avoid loss of volatiles, provide control release and greater stability of the encapsulated material (Eghbal & Choudhary, 2018; Araújo et al., 2020; Tang, Scher, Jeoh, 2020). In essential oils, for example, it was effective in retaining β-caryophyllene in black pepper (Bastos et al., 2020), linalool in jasmine (Lv et al., 2014), carvacrol and thymol in *Zataria multiflora* (Khatibi et al., 2021). The complex coacervation involves the electrostatic interaction between polymers with opposite charges (usually a protein and a polysaccharide), causing phase separation. Some important points must be considered in the coacervation process, such as pH, nature of the encapsulated material, core/wall material proportion, and proportion between polymers (Rojas-Moreno et al., 2018b; Tavares & Noreña, 2020; Khatibi et al., 2021). These factors are fundamental for the stability of the capsule obtained, which includes maintaining or enhancing biological activity, core retention, morphology, and suitable size for its application (Rojas-Moreno et al., 2018a).

The well-known biopolymers pair gelatin and gum arabic can be used with essential oils, and it is often used for studies on complex coacervation as a reference system, because their performance is predictable and it provides suitable spherical-shaped microcapsules (Dong et al., 2011; Lv et al., 2013; Khatibi et al., 2021). Gelatin is a biocompatible, biodegradable, easily available, and soluble protein at body temperature, thus presenting ideal conditions for pharmaceutical and food applications (Bonilla et al., 2018). Gum arabic is a natural gum, with emulsifying properties over a wide pH range (Isobe et al., 2020). This gum is an anionic complex polysaccharide, which has no taste and odor, so it does not change these characteristics in the system where it is added (Patel & Goyal, 2015). Therefore, the use of mixtures of gelatin and gum arabic appears to offer a good balance between cost and efficiency.

Campomanesia adamantium is a species found in the Brazilian Cerrado biome and presents essential oil in the various parts of the plant (Stefanello et al., 2008; Viscardi et al., 2017; Sá et al., 2018). Its fruits are commonly known as guavira, consumed fresh and the pulp is the part of the fruit that is much appreciated in the production of juices, jams, ice cream, and other preparations. During the pulping process, the residues formed by peel and seed represent approximately 40% of the whole fruit (Alves et al., 2020), which represents the mass of the fruit that is

generally not consumed or reused. These non-edible parts, which still have a peculiar aroma, can be used to extract essential oils, rich in terpenes and other bioactive compounds. The transformation of residues into new products for use in different industrial sectors allows adding value to them and reduce environmental problems caused by their direct disposal in nature (Alves et al., 2020).

A study carried out in our research group demonstrated that EO from guavira peel and seed has anti-inflammatory and antinociceptive activities (Viscardi et al., 2017). Given the above, the objective of the study was to obtain microcapsules of the essential oil obtained from guavira residues by the complex coacervation method and to establish the best encapsulation condition for the system. For this, the influence of the amount of core (essential oil) and proportion of gelatin to arabic gum as wall material was verified in relation to the antioxidant capacity, microencapsulation yield, and microcapsule morphology. Additionally, the antioxidant capacity and the chemical composition of the microencapsulated EO were compared with pure EO, as well as EO retention rate in the microcapsules.

2. Material and Methods

2.1 Reagents

Gum arabic, ethanol and bovine gelatin were acquired from *Dinâmica Química Contemporânea Ltda* (Diadema/SP, Brazil). Sodium acetate trihydrate, butyl hydroxytoluene, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and ferrous sulfate heptahydrate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Glacial acetic acid, n-hexane, and ferric chloride hexahydrate were obtained with analytical grade P.A.

2.2 Plant Material

Ripe fruits of guavira (*Campomanesia adamantium* Cambess. O. Berg) were acquired in the city of Ponta Porã, state of *Mato Grosso do Sul*, Brazil (22 ° 32' 09" S, 55 ° 43' 33" W). A voucher specimen is deposited in the herbarium of the Faculty of Biological Sciences of the Federal University of Grande Dourados - UFGD - Dourados/MS/Brazil (DDMS 4602 - Sisgen n ºA37EC3E).

2.3 Essential Oil Extraction

The residue (peel and seed) from the pulping of guavira was dehydrated at 50 ℃ for 20 h in a convective dehydrator designed by the research group, with an airflow of 0.5 m s^{-1}. The fruit fractions (pulp, *in natura* residue, dehydrated residue, crushed residue) are shown in **Figure 1.** The dehydrated residue was crushed in a multiprocessor (Mondial® Turbo Pratic MP-16-B, Bahia, Brazil) and EO extraction was performed by hydrodistillation in a Clevenger-type apparatus for 150 min (Viscardi et al., 2017). The removed EO was stored at -18 ℃ until use. The extracted EO yield (EOY) was calculated using the following formula: EOY (%) = (m_{EO}/m_{GR}) x 100, where m_{EO}: is the mass of extracted essential oil (g), and m_{GR}: is the mass of crushed dehydrated guavira residue (g).

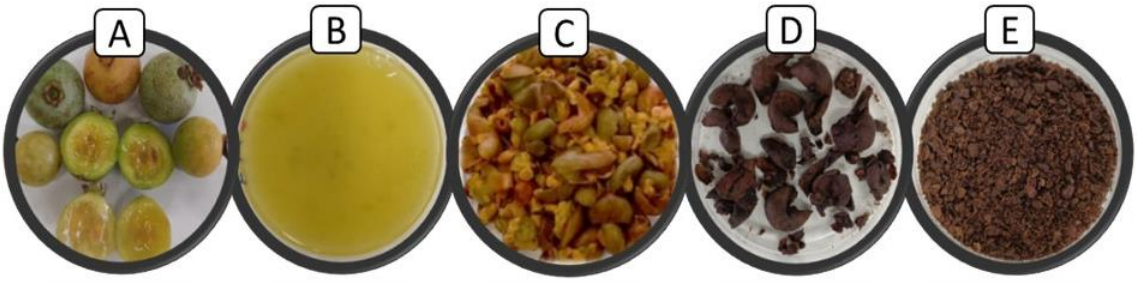

Figure 1. Guavira fruit (*Campomanesia adamantium*) and its fractions

A. External and internal appearance of the fruit. **B.** Pulp. **C.** Natural residue. **D.** Dehydrated residue. **E**. Crushed residue

2.4 Essential Oil Composition

The samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) with the use of Shimadzu GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) systems, according method used by Viscardi et al. (2017) and Cardoso et al. (2022). Chromatographic separation was performed using a DB-5 column (J & W Folsom, California) with 5% phenyl-dimethylpolysiloxane (30 m long x 0.25 mm diameter x 0.25 mm film thickness) in the following conditions: helium (99.999%) at a flow rate of 1 mL min^{-1}; and 1 µL of injection in the

ratio 1:10. Column temperature started at 50 ℃ for 5 min, heating at 3 ℃/min to 280 ℃. The injector, transfer line injection and detector were maintained at 250, 290, and 290 ℃, respectively. The MS scan parameters included 70 V ionization voltage and 50 to 600 Da mass change over a 0.3 s interval. Retention indices were calculated using a mixture of a homologous series of *n*-alkanes (C_8-C_{40}). The identification of the components was obtained by comparing the mass spectra of the samples with the spectra available in the NIST21 and WILEY229 libraries (Adams, 2007). The relative concentrations of the components were obtained by normalizing peak areas (%). Relative areas consisted of the average of triplicates.

2.5 Essential Oil Microencapsulation

The process used to obtain the microcapsules (MC) by complex coacervation was performed according to the literature (Gallo, 2019), with modifications (**Figure 2**). Gelatin (G) and gum arabic (GA) solutions (1%) were prepared with deionized water. Gelatin solution heated to 50 ℃ was mixed with EO in an Ultra-Turrax (digital IKA® T25) at 13400 rpm for 3 min, obtaining an emulsion. To this emulsion, GA solution heated to 50 ℃ and under agitation (800 rpm) was added, using a stirring/heating plate (IKA® - WERKE). Then the pH was adjusted to 4.0 ± 0.05 with glacial acetic acid (50%). The mixture was cooled to 10 ℃ ± 1 ℃ under agitation (250 rpm) and was kept to 10 ℃ for 24 h for complete deposition of the coacervate. The coacervate was frozen in an ultra-freezer at -60 ℃ (CL 120 Cold Lab, Brazil) and freeze-dried in a benchtop freeze dryer (Liotop, Liobras, model L101, São Carlos/SP, Brazil) at -50 ℃ for 96 h, the vacuum pressure of 30 μHg, obtaining the dry MC. Control MC were prepared without EO.

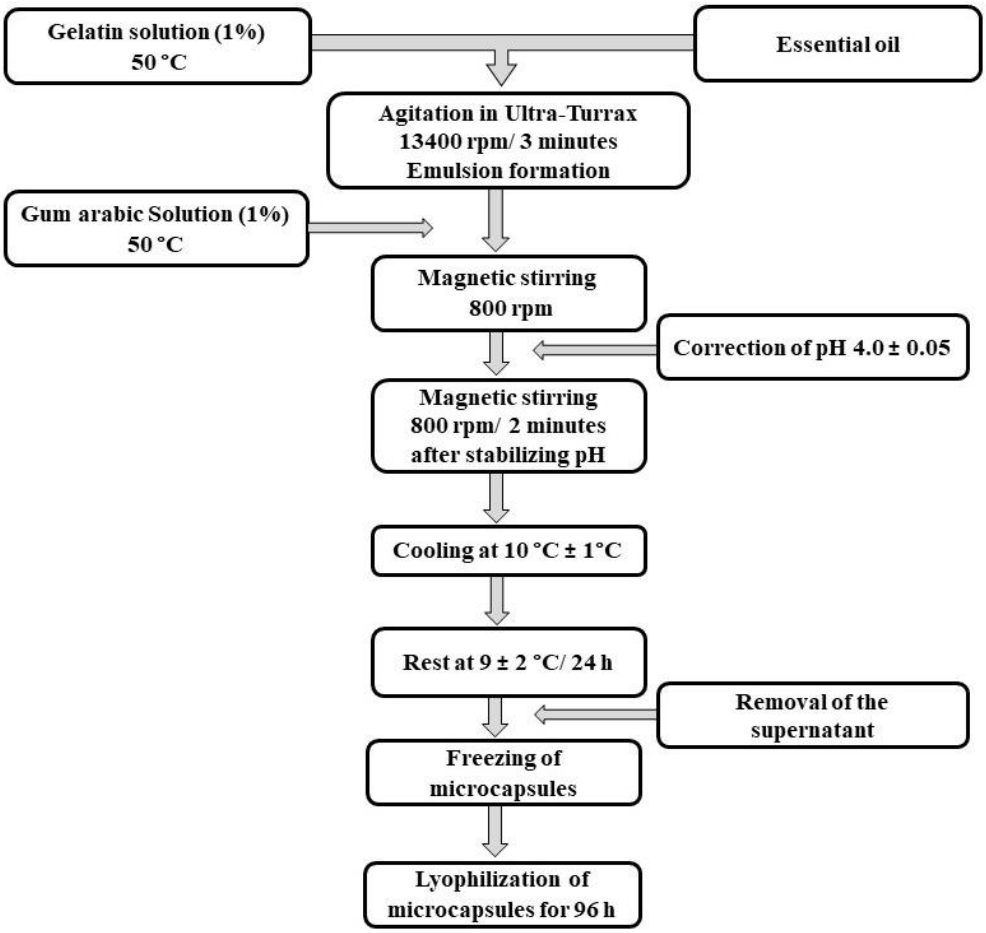

Figure 2. Flowchart of the microencapsulation process of guavira residue essential oil by complex coacervation followed by freeze drying, using gelatin and gum arabic as wall materials

2.5.1 Experimental Design Analyzes

The optimization of the process of microencapsulation was conducted using Response Surface Methodology coupled with a Central Composite Rotational Design (CCRD) 2^2, with five levels of each variable (**Table 1**). It

was carried out to verify the effects of the x_1: wall material (W) ratio formed by gelatin and gum arabic (G:GA = 1:1; 1:2; 1:3; 2:1 e 3:1), and x_2: mass of EO in the formulation of microcapsules (equivalent to: 20 - 42.8%). The parameters were established based on the literature (Khatibi et al., 2021). Eleven formulations were performed, including two repetitions at the central point, having as dependent variables: microencapsulation yield (MY), morphology, and antioxidant capacity (AC) of MC. The analysis methodologies for these variables are described below. Formulations of the CCRD were repeated twice and the microencapsulation procedure followed according to 2.5. Analyses were carried out in triplicate.

Table 1. Conditions for preparing essential oil microcapsules and their variation levels used in the central composite rotational design (CCRD)

Independent variables	Variation levels				
	-1.41 (-α)	-1	0	+1	+1.41 (+α)
G mass (g) (x_1)	0.5	0.65	1.0	1.35	1.5
G: GA ratio (w/w) equivalent to x_1	(1:3)	(1:2)	(1:1)	(2:1)	(3:1)
Essential oil mass (g) (x_2)	0.5	0.65	1.0	1.35	1.5
Essential oil (%) equivalent to x_2	20	24.5	33.3	40.3	42.8

G: gelatin; GA: gum arabic; EO: essential oil; w/w: mass/mass. x_1 and x_2: coded levels of the independent variables. x_1 is added to the constant mass of 2 g of total wall material. x_2 is added to the constant mass of 2 g of total wall material

Response surfaces were generated to verify the tendency to maximize microencapsulation yield and antioxidant capacity. The following polynomial equation (**Equation 1)** was fitted to data:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (1)$$

Where, b_n are constant regression coefficients, y is the response, and x_1, and x_2 are the coded independent variables.

2.5.1.1 Microencapsulation yield

The microencapsulation yield (MY) was calculated using the following formula: MY (%) = (m_{DR} / m_T) x 100 (Rutz et al., 2016), where m_{DR} and m_T are the mass of freeze-dried microcapsules (g) and the total mass of G + GA + EO of the formulation (g), respectively. In the control microcapsules, m_T is the mass of G and GA (g).

2.5.1.2 Antioxidant capacity

Antioxidant capacity (AC) was determined in the pure essential oil and microencapsulated essential oil (MEO), by method of the iron reduction (FRAP - Ferric Reducing Antioxidant Power) (Oliveira et al., 2019). To determine AC of the MEO, the MC's wall was first broken to release the EO (Yang et al., 2014). For this, 0.5 g of MC was weighed and 20 mL of ethanol was added. The mixture was vortexed for 2 min and immersed in an ultrasonic bath for 30 min. It was centrifuged (Rotanta 460, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 13,640 x*g* (10,000 rpm) for 15 min, and the supernatant was transferred to a 25 mL volumetric flask, completing the volume with ethanol PA.

For the FRAP assay, a solution of FRAP reagent was prepared by mixing 25 mL of sodium acetate buffer (0.3 M pH 3.6), 2.5 mL of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution (10 mM) and 2.5 mL of ferric chloride (20 mM). The sample (90 µL) obtained from dilutions of pure and MEO was mixed with distilled water (270 µL) and with 2.7 mL of FRAP reagent and incubated at 37 ℃ for 30 min in the dark. Absorbance was measured at 595 nm in a spectrophotometer (Libra S60PC, Biochrom, England) using the reducing solution (FRAP reagent) as "zero" of the assay. Ethanol PA was used as a negative control. A calibration curve was plotted using $FeSO_4$ in the range 0.5-2.0 mmol L^{-1} concentration with absorbances at 595 nm ($y = 0.0126x - 0.075$; $R^2 = 0.9997$), and the curve was used to calculate the sample's AC. Results were expressed in µmol $FeSO_4$ g^{-1}.

2.5.1.3 Morphology

The morphology of the wet and freeze-dried microcapsules was analyzed by optical microscopy and scanning electron microscopy (SEM), respectively. It was used an optical microscope (Nikon Eclipse E200, Japan) with magnification of 100x and 400x coupled with a photographic camera (Moticam 2300, 3M Pixel, Nikon, Japan) using Motic Image Plus software. For SEM analysis, was used a JEOL microscope, model JSM-6380LV (Tokyo,

Japan). The MC was accommodated on double-sided carbon tape, which was fixed on aluminum stubs and metalized with a thin layer of gold. The images were captured with voltage acceleration of 15 kV at a working distance of 11 mm and magnified at 800x.

2.5.2 Composition of Microencapsulated Essential Oil

EO extraction from MC was performed according by Campelo et al., (2017) with some modifications. MC were treated with hexane (50 mg mL^{-1}) and submitted to an ultrasonic bath for 10 min. After this period, the mixture was left to stand for phase separation and the organic fraction (supernatant) containing the EO was collected. The solvent was evaporated in a fume hood. After drying, the sample was solubilized in hexane, obtaining a final concentration of 0.2 mg mL^{-1}. The procedure for analysis of the MEO composition was according to item *2.4.*

2.5.3 Essential Oil Retention Rate in Microcapsules

The EO retention rate (RR) in the MC was calculated from the sum of the compounds identified by gas chromatography in the EO extracted from the microcapsule (M_e), in relation to the EO incorporated at the beginning of the process (M_i), according to Locali-Pereira et al., (2020), using the following formula: RR (%) = (M_e / M_i) x 100.

2.6 Statistical Analysis

The results of the CCRD were evaluated by the 5% confidence level, coefficient of determination (R^2), and Fisher's test value (F value) obtained from the analysis of variance (ANOVA) (Rodrigues, Iemma, 2005), using Statistica 8.0 software (StatSoft, 2007). All chemical analyses were performed in triplicates and the CCRD design experiments were repeated twice consecutively (Rodrigues, Iemma, 2005). Results of the microencapsulation yield of control freeze-dried microcapsules were submitted to analysis of variance (ANOVA) followed by Tukey's mean comparison test ($p < 0.05$). All results were presented by the mean value of replicates and standard deviation (SD).

3. Results and Discussion

3.1 Essential Oil Yield

The essential oil yield from dried guavira residue was 0.37 ± 0.08% (m m^{-1}). Considering that peels and seeds represented on average 55% of the fresh fruit, to obtain 100 g (equivalent to 112 mL) of EO, approximately 150 Kg of whole fruit or 27 Kg of dry residue are required. In most scientific studies, the EO content extracted from aromatic plants is below 1% (Oliveira et al., 2019; Jesus et al., 2020).

In previous study, the EO yield was presented separately for seeds (0.98% m m^{-1}), and peel (0.32% m m^{-1}) of *C. adamantium* (Viscardi et al., 2017). In the extraction of essential oils from fruit peels of 3 species of citrus (*Citrus deliciosa*, *Citrus sinensis*, and *Citrus reticulata* - Myrtaceae), was obtained 0.8% yield (Dias et al., 2020). Despite the apparent low yield, the EO industry is expanding and the discovery of new sources of EO is economically important due to the biological potential favored by the extraction of EO from underused waste and due to high market value (Bueno et al., 2021; Bin et al., 2022). Thus, the percentage of EO obtained from residues of the pulping of guavira is relevant, which are often discarded in the environment, generating an increase in waste. In addition, its disposal represents the underutilization of a source of several bioactive compounds.

3.2 Chemical Composition of Pure Essential Oil

Twenty-eight constituents were identified in the EO extracted from the residues of the peel + seed fraction of the guavira (**Table 2**). Based on the retention index, the major components identified were three monoterpenes limonene (18.59%), α-pinene (17.53%), β-ocimene (11.13%), and one sesquiterpene, the β-caryophyllene (9.82%). Viscardi et al. (2017) identified a predominance of limonene (13.07%) and thujopsene (6.96%) in the composition of the EO extracted from the peel of guavira fruits, and limonene (20.89%) and β-pinene (11.48%) in the EO extracted from the seeds. Limonene is a major component in the fruits of the genus *Campomanesi*a (Marin et al., 2008; Viscardi et al., 2017). The elucidation of the compounds contained in the natural products is of extreme importance for the knowledge of its chemical and biological properties. Studies point out that limonene is very aromatic and has biological properties as anti-inflammatory (Viscardi et al., 2017; Yu et al., 2017), antitumor (Magalh ães et al., 2020; Weimer et al., 2021), antifungal (Dias et al., 2020), neuroprotective (Eddin et al., 2021), and antioxidant (Yu et al., 2017; Weimer et al., 2021). The EO of guavira residues is also composed of α/β-pinenes, that are very aromatic and have gastroprotective, antioxidant, cytoprotective, cytogenetic, and anticonvulsant effects (Salehi et al., 2019). β-ocimene presents antileishmanial activity (Sousa et al., 2023). The β-caryophyllene was the major sesquiterpene identified in this EO, which has antioxidant activity (Nogueira Sobrinho et al., 2020), and inhibits cell proliferation (Irrera et al., 2020).

Table 2. Major components identified in the essential oil extracted from the guavira pulping residue

Components	RI_L	RI_E	(%)*
Monoterpenes Hydrocarbons			
α-pinene	939	939	17.53 ±0.17
β-pinene	979	980	3.50 ±0.02
Myrcene	991	991	1.66 ±0.01
α-phellandrene	1009	1009	2.11 ±0.05
Limonene	1029	1029	18.59 ±0.20
β-phellandrene	1031	1031	1.86 ±0.02
β-ocimene	1037	1037	11.13 ±0.19
Terpinolene	1088	1088	3.13 ±0.11
γ-Terpinene	1054	1054	1.22 ±0.01
Oxygenated Monoterpenes			
1,8-cineole	1031	1031	3.99 ±0.12
α-terpineol	1189	1189	2.72 ±0.05
Sesquiterpenes Hydrocarbons			
δ-elemene	1339	1339	1.21 ±0.03
α-cubebene	1351	1351	1.16 ±0.01
Longicyclene	1366	1366	1.41 ±0.03
α-Ylangene	1373	1374	0.38 ±0.03
α-copaene	1377	1377	2.93 ±0.05
β-elemene	1391	1391	1.74 ±0.01
Longifolene	1402	1402	1.07 ±0.02
β-caryophyllene	1404	1404	9.82 ±0.08
Aromadendrene	1465	1465	1.63 ±0.03
Germacrene D	1485	1485	3.01 ±0.01
Biciclogermacrene	1500	1500	0.68 ±0.01
β-Bisabolene	1505	1501	0.79 ±0.01
δ-cadinene	1514	1514	0.85 ±0.01
Germacrene B	1561	1561	0.95 ±0.01
Oxygenated sesquiterpenes			
Spathulenol	1578	1578	2.86 ±0.05
β-Acorenol	1632	1632	1.17 ±0.03
α-Muurolol	1644	1644	0.90 ±0.02
Total			100.00

*Values (%) represent mean ± SD

RI_E = experimental retention index; RI_L = literature retention index

The composition of EO can be influenced by different factors, such as the part of the plant used, harvest season, soil type, vegetative cycle, and climatic conditions, that influence in the formation, concentration and types of secondary metabolites (Sá et al., 2018; Dudeja et al., 2023) . The variability of the chemical composition of EO can provide information about their biological properties (Weimer et al., 2021). On the other hand, this variability makes it difficult to relate the biological activity with the individual components of the EO (Sá et al., 2018). Therefore, beneficial effects are often associated with the synergistic effect between the major and minor components of the essential oils.

3.3 Analysis of Central Composite Rotational Design for Microencapsulation Yield and Antioxidant Capacity

The experimental conditions and the results of 11 assays more the control formulations are shown in **Table 3** and are discussed below. The data were submitted to analysis of variance ANOVA (**Table 4)** and indicated that microencapsulation yield (MY) was directly affected by the ratio of G:GA encapsulants (x_1) and AC was affected by amount of inserted EO (x_2).

Table 3. Values of the independent and dependent variables of the central composite rotational design for guavira essential oil microcapsules, control microcapsules, and pure essential oil

	x_1	x_2		Dependent variables*	
Formulations	G:GA ratio	Essential Oil (g)	Essential Oil (%)	MY (%)	Antioxidant capacity (μmol $FeSO_4$ g^{-1} MEO)
F1	(1:2)	0.65	24.5	55.85 ± 5.1	113.91 ± 11.4
F2	(1:2)	1.35	40.3	67.93 ± 2.7	181.58 ± 8.4
F3	(2:1)	0.65	24.5	68.90 ± 5.7	102.31 ± 0.0
F4	(2:1)	1.35	40.3	67.06 ± 5.5	172.71 ± 0.3
F5	(1:3)	1.0	33.3	24.00 ± 4.2	134.20 ± 6.4
F6	(3:1)	1.0	33.3	51.21 ± 5.5	133.73 ± 6.0
F7	(1:1)	0.5	20.0	79.77 ± 0.6	28.29 ± 0.0
F8	(1:1)	1.5	42.8	79.58 ± 6.2	173.93 ± 13.4
F9 (C)	(1:1)	1.0	33.3	82.68 ± 2.7	163.57 ± 14.1
F10 (C)	(1:1)	1.0	33.3	78.92 ± 2.4	152.88 ± 9.4
F11 (C)	(1:1)	1.0	33.3	79.52 ± 1.7	151.08 ± 11.3
Control microcapsules					
Control formulations (without oil)	1:1	-	-	77.28 ± 1.13[a]	-
	1:2	-	-	42.73 ± 1.35[d]	-
	1:3	-	-	21.19 ± 1.95[e]	-
	2:1	-	-	71.19 ± 2.41[b]	-
	3:1	-	-	51.50 ± 3.96[c]	-
Pure EO					
Essential oil	-	-	-	-	183.30 ± 3.8

* Expressed as the mean value ± standard deviation.

Control microcapsules: different superscript letters indicate a statistically significant difference ($p < 0.05$) by Tukey's test.

$FeSO_4$: ferrous sulphate; F: formulation; G: gelatin; GA: gum arabic; EO: essential oil; MEO: microencapsulated essential oil; MY: microencapsulation yield; F9, F10 and F11 (C): represent the center point

Table 4. Analysis of variance in relation to the parameters of microencapsulation yield and antioxidant capacity of microcapsules containing essential oil from guavira residues

	Sum of Squares	*Df*	Mean Square	F-value	*p*-value	R^2
Microencapsulation Yield						0.9404
(x_1) (G:GA) (L)	339.29	1	339.29	83.149	0.011	
(x_1) (G:GA) (Q)	2203.44	1	2203.44	539.99	0.001	
(x_2) EO (L)	15.47	1	15.47	3.79	0.190	
(x_2) EO (Q)	10.44	1	10.44	2.55	0.251	
Lack of fit	176.26	3	58.75	14.40	0.065	
Pure error	8.16	2	4.08			
Total SS	3092.84					
Antioxidant Capacity						0.9197
(x_1) (G:GA) (L)	57.29	1	57.29	1.257	0.379	
(x_1) (G:GA) (Q)	175.48	1	175.48	3.849	0.189	
(x_2) EO (L)	12610.17	1	12610.17	276.62	0.003	
(x_2) EO (Q)	1773.24	1	1773.24	38.899	0.025	
Lack of fit	1461.92	3	487.31	10.69	0.086	
Pure error	91.17	2	45.59			
Total SS	19355.60					

Significant when *p-value* ≤ 0.05. 2 factors, 1 block, 11 runs. (L) = linear; (Q) = quadratic; G = gelatin; GA = gum arabic; EO = essential oil; SS = sum of squares; R^2 = determination coefficient; D_f = degrees of freedom

The analysis of variance showed that the ratio of G:GA significantly influenced ($p < 0.05$) the MY, both in linear and quadratic interactions (**Table 4**), presenting a coefficient of determination (R^2) of 0.940. A value of $R^2 > 0.75$ is indicated for developing a statistical model for optimization (Peng et al., 2020). Furthermore, the "*Lack of Fit*" presented *p*-value of 0.065, not significant for this model. Thus, **Equation 2** was obtained, which represents the predictive second-order model as a function of the studied variable.

$$MY\ (\%) = -117.00 + 373.06x_1 - 0.10x_2 - 159.27x_1^2 + 0.02x_2^2 - 1.11x_1x_2 \quad (2)$$

Once the model fits the experimental data, the response surface plot is displayed, which shows the influence of each variable (G:GA ratio and amount of oil) on MY (**Figure 3A**). The 3-dimensional response surface plots reinforce the results presented in **Table 3**. Higher MY (greater than 78%) was observed with G:GA 1:1 ratio (F7, F8, F9, F10, and F11 formulations), and lower MY occurred in the 1:3 ratio (F5), indicating the importance of suitable G:GA ratio in the formation of microcapsules, regardless of the amount of EO.

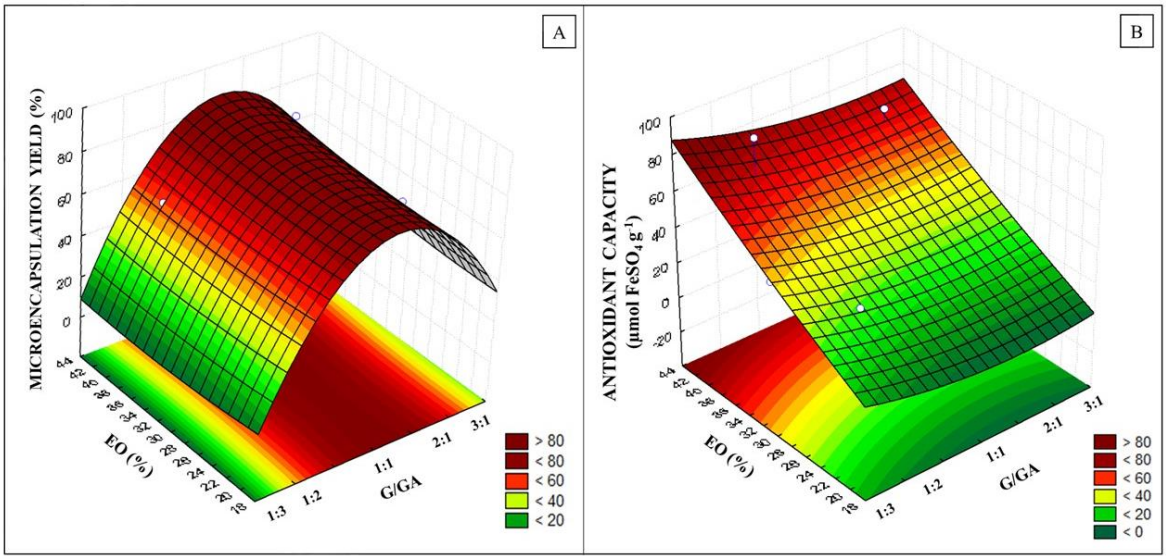

Figure 3. Response surface graph for effects of wall material ratio (G:GA) and essential oil amount (%) on microencapsulation yield (A) and antioxidant capacity (B)

The proportion of encapsulants interfered in the MY since it changed the electrostatic interaction between the wall materials, which is essential to complex coacervation process. According to Eghbal & Choudhary, (2018), the interaction usually occurs between a positively charged protein and a negatively charged polysaccharide, where proteins have positive charges when they are at a pH below their isoelectric point (pI) and negative charges above it. In the present study, in relation to MY, pH 4.0 was more effective when the ratio was 1:1 using the combination of gelatin and gum arabic. Other authors show the suitability of pH 4.0 on the formation of microcapsules and on the interaction of G with GA in a 1:1 ratio, such as in the encapsulation of essential oil from *Zataria multiflora* (Khatibi et al., 2021), *Vetiveria zizanoides* (Prata et al., 2008), and of the thyme (Gonçalves et al., 2018). The formation of microcapsules was also verified by the control assays (without oil), which showed a yield with a significant difference ($p < 0.05$) among all tests, ranging from 21.19% (G:GA 1:3) to 77.28% (G:GA 1:1), according to **Table 3**. These results were consistent with the formulations containing essential oil.

Antioxidant capacity was assessed after rupture of the MC to release the EO, and AC has been indicated as µmol FeSO4 g^{-1} MEO (**Table 3**). Analysis of variance (**Table 4)** indicated that only the amount of EO significantly influenced ($p < 0.05$) both the linear and quadratic interaction with $R^2 = 0.919$. Predictive second-order model was obtained (**Equation 3**) for this variable, as well as the response surface plot (**Figure 3B**) to show the influence of each variable on AC.

$$AC = -330.84 + 71.0x_1 + 22.81\,x_2 - 44.95x_1^2 - 0.28x_2^2 + 0.35x_1x_2 \quad (3)$$

There is a significant increase of the AC with amount of EO, being not significant for the encapsulants ratio. The highest AC was from F2 (181.58 µmol FeSO4 g^{-1} MEO), followed by F8 and F4 (173.96 and 172.71 µmol $FeSO_4$ g^{-1} MEO, respectively). In these formulations, only F8 has the same G:GA ratio (1:1) that had shown the highest MY. On the other hand, F2 and F4 were prepared with the same EO mass (40.3%), and with a G:GA ratio of 1:2

and 2:1, respectively. Although the G:GA ratio was not a statistically significant variable in AC, the influence of the wall formed in the microcapsule on core protection can be seen. This was noted when we compared the formulations with the same amounts of EO, but with a different G:GA ratio (F1 versus F3, and F2 versus F4). In the F2 formulation, besides containing 40.3% of EO, GA is in greater quantity in relation to G. This condition seems to change the electrostatic interaction strength with gelatin, leads to formation of a thicker microcapsule wall, favoring the high retention of the AC (**Table 3**), because it may increase microcapsule stability and lead to slower release of encapsulated EO (Alves et al., 2014). In the formulation F4, with the highest mass of gelatin (G:GA 2:1), it is possible that the microcapsule wall is not strong enough to keep volatile compounds due to the porosity of the wall. The premature release of encapsulated substances and loss of bioactives can be attributed, in part, to the formation of small ice crystals in the frozen emulsion which, during the freeze-drying process, promote the formation of pores in the microparticles, weakening the core protection (Tavares & Noreña, 2020).

Considering the incorporation of EO in the MC formulations and the percentage of EO retention (**Table 5**), the AC values were compared with the result of the AC of pure EO (183.30 μmol $FeSO_4$ g^{-1}), which presented 99.0, 94.9, and 94.2% (F2, F8, F4, respectively) equivalent to pure oil (**Table 3**). These results indicate that there was no significant reduction in AC during the stages of the microencapsulation process by complex coacervation, using gum arabic, gelatin and EO in the concentrations and proportions of those that were considered the best MC formulations.

Table 5. Constituents identified in encapsulated essential oil according to the percentage of incorporated essential oil in each formulation

	Formulations (%)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
	EO added in each formulation (%)								
Compounds (%)	24.5	40.3	24.5	40.3	33.3	33.3	20	42.8	33.3
α-pinene	4.23 ±0.08	7.06 ±0.07	4.51 ±0.03	7.08 ±0.06	5.59 ±0.06	5.67 ±0.05	3.38 ±0.03	7.21 ±0.04	5.81 ±0.04
β-pinene	0.87 ±0.01	1.40 ±0.02	0.93 ±0.01	1.42 ±0.02	1.14 ±0.02	1.17 ±0.02	0.71 ±0.02	1.45 ±0.01	1.21 ±0.02
Limonene	3.99 ±0.09	6.74 ±0.07	4.42 ±0.05	6.76 ±0.05	5.51 ±0.07	5.45 ±0.03	3.22 ±0.04	6.99 ±0.04	5.67 ±0.03
1,8-cineole	0.89 ±0.01	1.47 ±0.03	0.98 ±0.01	1.45 ±0.03	1.23 ±0.02	1.24 ±0.02	0.77 ±0.01	1.51 ±0.01	1.30 ±0.02
β-ocimene	2.41 ±0.06	4.04 ±0.05	2.66 ±0.02	4.12 ±0.11	3.29 ±0.04	3.23 ±0.01	1.91 ±0.02	4.21 ±0.03	3.29 ±0.01
Terpinolene	0.81 ±0.01	1.41 ±0.05	0.91 ±0.01	1.47 ±0.04	1.07 ±0.02	1.11 ±0.02	0.66 ±0.02	1.41 ±0.01	1.16 ±0.02
α-terpineol	0.69 ±0.01	1.17 ±0.05	0.80 ±0.01	1.34 ±0.07	0.95 ±0.02	0.99 ±0.02	0.55 ±0.01	1.18 ±0.01	1.01 ±0.02
α-copaene	0.71 ±0.01	1.15 ±0.01	0.83 ±0.01	1.33 ±0.05	0.98 ±0.02	1.01 ±0.02	0.59 ±0.01	1.24 ±0.01	1.03 ±0.02
β-caryophyllene	2.23 ±0.03	4.01 ±0.07	2.53 ±0.02	4.03 ±0.03	3.21 ±0.01	3.08 ±0.02	1.78 ±0.01	4.07 ±0.04	3.17 ±0.01
Germacrene D	0.66 ±0.01	1.04 ±0.03	0.78 ±0.01	1.04 ±0.02	0.91 ±0.01	0.93 ±0.01	0.51 ±0.01	1.10 ±0.01	0.98 ±0.02
Spathulenol	0.84 ±0.01	1.54 ±0.02	0.91 ±0.01	1.61 ±0.02	1.09 ±0.02	1.11 ±0.02	0.68 ±0.01	1.39 ±0.01	1.18 ±0.02
Sum of constituents (%)	18.33	31.03	20.26	31.65	24.97	24.99	14.76	31.76	25.81
EO retention in MC (%)	74.82	76.99	82.7	78.53	74.92	74.98	73.8	74.21	77.44

Formulations 1 - 9 have different proportions between the wall materials and the amount of incorporated essential oil: F1 (G:GA 1:2 and EO:24.5%); F2 (G:GA 1:2 and EO:40.3%); F3 (G:GA 2:1 and EO:24.5%); F4 (G:GA 2:1 and EO:40.3%); F5 (G:GA 1:3 and EO:33.3%); F6 (G:GA 3:1 and EO:33.3); F7 (G:GA 1:1 and EO:20%); F8 (G:GA 1:1 and EO:42.8%); F9 (G:GA 1:1 and EO:33.3%). G = gelatin; GA = gum arabic; EO = essential oil; MC = microcapsules.

The AC of EO from guavira residues is influenced by its chemical composition. It is suggested that the AC of guavira EO can be attributed to the whole of constituents or to β-caryophyllene since this compound showed good antioxidant potential with a low IC_{50} value (42 μg mL^{-1}; 98 μg mL^{-1}) by the β-carotene/linoleic acid co-oxidation and DPPH methods, respectively (Nogueira Sobrinho et al., 2020). The antioxidant activity of EO can occur due to the action of a major compound that has the biological potential, or due to the synergistic effect (Nascimento et al., 2018; Valarezo et al., 2022). Thus, the EO extracted from guavira residue can be used synergistically, for example, partially replacing a synthetic antioxidant or adding this activity to other potentialities, since an *in vivo* study has already indicated anti-inflammatory and analgesic activity with the EO of this same type plant matrix (Viscardi et al., 2017).

Considering the encapsulation yield and the antioxidant capacity analyzed, from the point of view of retaining of the pure EO antioxidant capacity, we observed that the proportion of the wall material that provides the greatest number of microcapsules is not always the one that retains the most bioactive compounds. The characteristics of

the oil to be encapsulated influence its AC and yield, such as oil type (fixed or volatile). In view of this and considering the retention of the antioxidant capacity of the MEO, the 1:2 ratio of G:GA was more effective. Thus, although the G:GA ratio of 1:1 provides higher microcapsules yield, part of them may be hollow or partially filled, which would explain the lower AC values.

3.4 Microcapsule Morphology

Figure 4 shows the optical microscopy images of wet microcapsules (larger images) and SEM of dry microcapsules (smaller images). The optical images of the G:GA 1:1, 1:2, 1:3, 2:1 e 3:1 control formulations (without EO) are showed in **Figure 4 - A, B, C**, **D and E**, respectively.

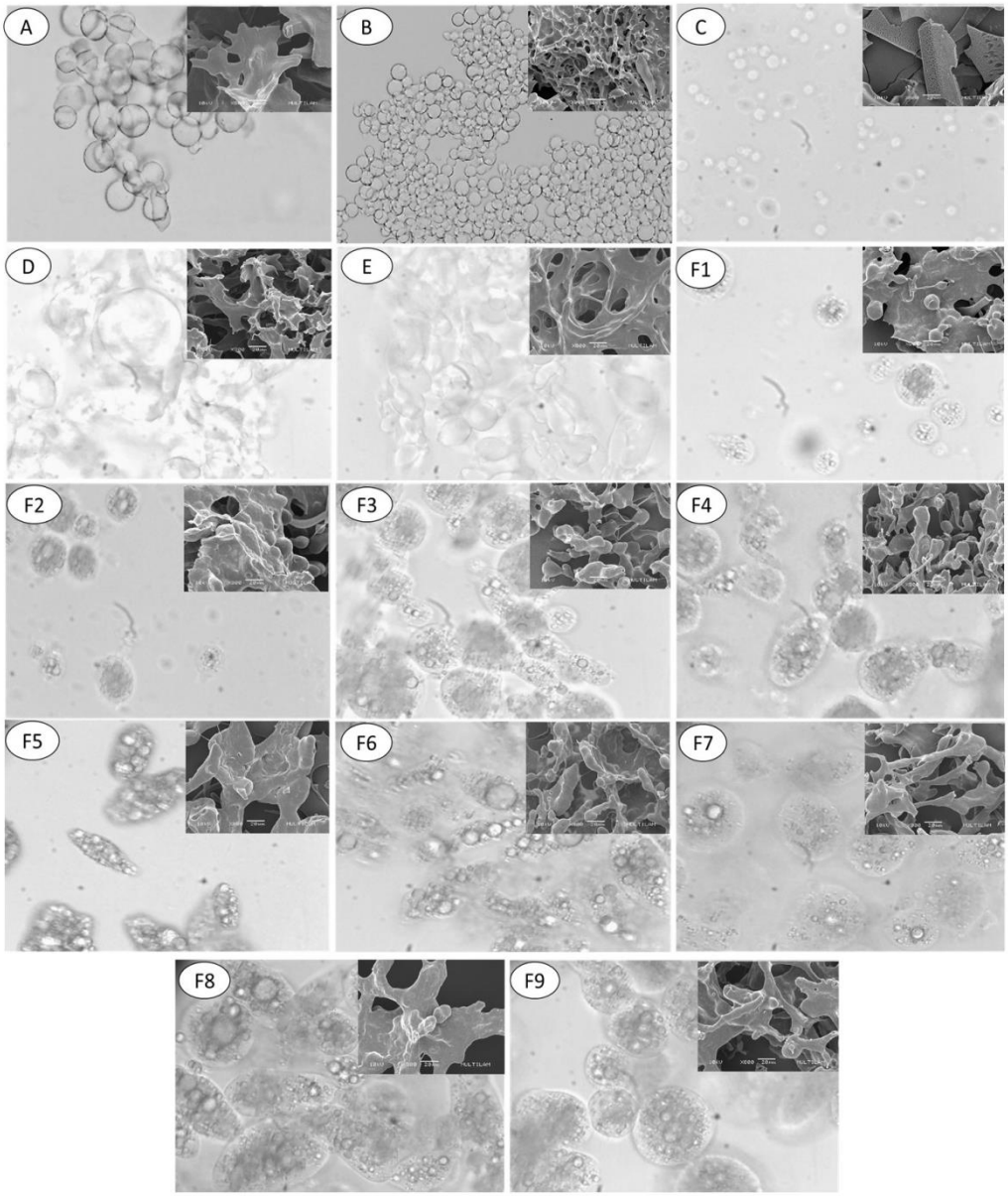

Figure 4. Optical microscopy (larger rectangle - x100 magnification) and scanning electron microscopy (smaller rectangles - x800 magnification) images of wet and freeze-dried microcapsules, respectively, obtained by complex coacervation

A-E: Control formulations, without EO (G:GA of 1:1, 1:2, 1:3, 2:1, and 3:1). **F1**: G:GA 1:2 and EO: 24.5%. **F2**: G:GA 1:2 and EO: 40.3%. **F3**: G:GA 2:1 and EO: 24.5%. **F4**: G:GA 2:1 and EO: 40.3%. **F5**: G:GA 1:3 and EO: 33.3%. **F6**: G:GA 3:1 and EO: 33.3%. **F7**: G:GA 1:1 and EO: 20%. **F8**: G:GA 1:1 and EO: 42.8%. **F9**/F10/F11: G:GA 1:1 and EO: 33.3%. *Abbreviations:* F = formulation; G = gelatin; GA = gum arabic; EO = essential oil

The morphological differences of the MC were dependent on the G:GA ratio and the amount of EO. The optical images of the G:GA 1:1, 1:2, and 1:3 control formulations (without oil) (**Figure 4 -A, -B,** and **-C)**, showed a spherical shape and well-formed wall. The SEM images of these MC indicated the presence of pores on their surface, similar to a sponge. This porous structure may be associated with the freeze-drying process, where water in the solid state (frozen) is evaporated by sublimation, leaving empty spaces (porous structure) and an irregular shape (Rutz et al., 2017). In the G:GA ratio of 1:2, microcapsules with smaller pores are observed in relation to the other proportions. Optical micrographs of the control formulations with a higher amount of gelatin compared to gum arabic (2:1 and 3:1) showed irregular morphology and many pores when verified in SEM images (**Figure 4 -D** and **-E)**.

A surface without cracks or pores is important to reduce the permeability of the microcapsule wall and protect the encapsulated EO (Khatibi et al., 2021). Crosslinkers are sometimes used to increase core protection in the microcapsule or improve its morphology (Lv et al., 2013; Rojas-Moreno et al., 2018a; Khatibi et al., 2021), but the addition of crosslinking agent was not always effective or necessary (Alvim & Grosso, 2010; Koupantsis & Paraskevopoulou, 2017). Paprika oil was encapsulated with gelatin and gum arabic, obtaining microcapsules with a spherical, multinucleated shape, maintaining the integrity of the wall even without the use of crosslinkers, when freeze-drying was used for drying after complex coacervation (Alvim & Grosso, 2010).

Higher proportions of gelatin in relation to gum arabic (**Figure 4 -F3, -F4,** and **-F6**) disturb the formation of spherical or oval MC and may represent a reduction in encapsulation efficiency, probably by weakening interactions between polymers (Zuanon et al., 2013; Rojas-Moreno et al., 2018b). This explains the lower AC from the F3 in relation to F1, and from the F4 in relation to F2, when they have the same amount of EO (**Table 3**). Spherical particles are desirable because they exhibit greater fluidity, lower surface/volume ratio, and better coverage of the encapsulated material (Badke et al., 2019).

MC containing EO showed multinucleated structures, typical of using high-speed homogenization when mixing EO to the wall material (Dong et al., 2011). All microcapsules had an agglomerated and sponge-like appearance. Similar morphology was reported in EO microencapsulated from *Zataria multiflora* using gelatin and gum arabic (1:1, 1:2 and 2:1) as wall materials by the complex coacervation/freeze drying process (Khatibi et al., 2021). These structures were also observed in other studies of microencapsulation by complex coacervation (Koupantsis & Paraskevopoulou, 2017; Rutz et al., 2017; Araújo et al., 2020) and indicate that the morphology of the microcapsule can be influenced by the combination of wall materials, by the material to be encapsulated, by the speed of homogenization during the emulsification process, and by the drying method of the MC (Rutz et al., 2017; Eghbal & Choudhary, 2018).

3.5 Chemical Composition and Retention Rate of Encapsulated Essential Oil

Table 5 shows the constituents of the different MC formulations (F1 - F9). Their percentages are considered in relation to the total EO inserted in each formulation. It is noted that the same major constituents in the EO remained mostly in the MC formulations. The sum of the identified constituents was compared with the percentage inserted of EO in each formulation of the MC, obtaining the retention rate (RR). EO retention in the MC (**Table 5**) was greater than 73% in all formulations. Results agree with other encapsulation studies by complex coacervation, such as the retention of sweet orange EO (52%), encapsulated with whey protein and gum arabic (Rojas-Moreno et al., 2018a) and calendula oil (79%) encapsulated with gelatin and gum arabic (Badke et al., 2019). The combination of gelatin and gum arabic is a classic system for complex coacervation, which has been used to retain EO (Khatibi et al., 2021).

The results of microencapsulation yield, antioxidant capacity, and MC morphology showed that the combinations of G:GA with the best results were 1:1 and 1:2, with incorporation above 33% of EO. These results help to define MC formulations of essential oil extracted from *C. adamantium* fruit residues, with retention of bioactive compounds and to propitiate their technological application.

4. Conclusion

Essential oil from *C. adamantium* fruit residues was for the first time efficiently microencapsulated using gelatin and gum arabic as wall materials by the complex coacervation method followed by freeze drying. The combination with more gum arabic in the formation of the microcapsule wall provided better results in formulations with more EO, representing major protection for the core material. The method was effective in retaining the major compounds of EO (limonene, α-pinene, β-ocimene and β-caryophyllene), therefore obtaining microcapsules with retention of biological activity in a sustainable process. Thus, the complex coacervation process for the system in

study proved to be easy and with potential applicability for the use of EO obtained from guavira fruit residues. The essential oil extracted from this matrix and the microparticles formulated from it showed antioxidant potential, suggesting applications in the food, pharmaceutical or cosmetic industries. Results add sustainable value to residue normally discarded, which enables better use of residue generated by the food industry. Further research is needed to study the practical applications of this encapsulation system on an industrial scale.

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Authors' contributions

Conceptualization: M.C.B., A.P.F.M., E.J.S.A.; Methodology: M.C.B., E.J.S.A.; Formal analysis and investigation: M.C.B., L.S.A., S.S.Y., D.A.G., C.A.L.C.; Data curation and Validation: M.C.B., D. M.S.V., E.J.S.A.; Resources: D.A.G., C.A.L.C., E.J.S.A.; Writing - original draft: M.C.B.; Supervision, Project administration and Funding acquisition: D.M.S.V., E.J.S.A. All authors commented on previous versions of the manuscript, read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no conflict of interest. They have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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