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CASHEW GUM-GALACTOMANNAN BLENDS FOR ROSEMARY ESSENTIAL OIL ENCAPSULATION

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ABSTRACT

Combinations of plant polysaccharides can result in a wall material with desirable and appropriate encapsulating characteristics. The aim of this study was to evaluate the influence of a novel matrix based on galactomannan and Cashew gum on the microencapsulation of rosemary essential oil by spray drying. First, an emulsion was prepared, and sodium trimetaphosphate was added to cross-link the polysaccharides. A central composite rotatable design was then used to optimize the proportion of polysaccharides needed for the formation of microparticles. An increase in galactomannan concentration significantly affected viscosity ($p < 0.05$), emulsion droplet size, and oil particle size. Higher retention and encapsulation efficiencies were observed for lower galactomannan concentrations. SEM photomicrographs showed no cracks or microparticle fissures. An appropriate percentage for the efficient encapsulation of rosemary essential oil was 19.4% (w/v) cashew gum and 0.4% (w/v) galactomannan.

Keywords: Cashew gum, Encapsulation, Essential oil, Galactomannan, *Rosmarinus Officinalis*.

INTRODUCTION

Rosmarinus officinalis L. (rosemary) is a well-known decorative plant, where the leaves are often used as a spice to flavor various foods, such as stuffing and roast meats, and the bioactive compounds are valued for their beneficial effects on human health (BABOVIC *et al.*, 2010). The rosemary essential oil extracted from its leaves by hydro distillation has antibacterial, antioxidant, and free radical-scavenging properties (MEZZA *et al.*, 2018; ALIZADEH *et al.*, 2019). However, the compounds present in the essential oil that are responsible for the aroma, flavor, and attributes of *R. officinalis* are volatile and unstable in the presence of oxygen, moisture, and heat. Therefore, microencapsulation is an alternative method for reducing the loss of bioactive components.

Encapsulation by spray drying is particularly advantageous because it is simple, economical, efficient, continuous, and applicable to various types of products. In the spray drying process, the wall material must not only protect the essential oil from oxidation, chemical interactions, or volatilization but also maximize the retention of the essential oil after the drying process (BOTREL *et al.*, 2012; CARMO; FERNANDES; BORGES, 2015). Various materials (carriers or coadjuvants) have been incorporated into foods to decrease the loss of active compounds and serve as encapsulating agents, including modified starches, gum arabic, and cyclodextrins (FERNANDES; CANDIDO; OLIVEIRA, 2012). Additionally, several biopolymers have been investigated in studies using spray-drying encapsulation, such as konjac gum for sweet orange oil (YANG; XIAO; DING, 2009), galactomannan for ascorbic acid (SOUZA *et al.*, 2015), and tara and xanthan gums for pitanga juice (RUTZ *et al.*, 2013). The biopolymer blends have been used to complement desirable characteristics, such as emulsifying properties and viscosity, of each molecule for encapsulation applications (ARRIOLA *et al.*, 2019; MENDES *et al.*, 2020; MORALES *et al.*, 2020). Encapsulation involving biopolymers is especially attractive because they are natural and generally show low toxicity.

Galactomannans are neutral polysaccharides composed of a linear chain of mannose residues connected by β (1 $\rightarrow$ 4) glycosidic bonds, to which galactose residues are linked by α (1 $\rightarrow$ 6) bonds. They are found in the endosperm of legume seeds and are commercially important, mainly as thickeners and stabilizing agents. These polysaccharides are non-toxic, biocompatible, and a source of fiber for products. The structural relationship between mannose and galactose is one of the main biochemical characteristics of galactomannans; in fact, the ratio of galactose/mannose affects the physicochemical properties of the polymer (e.g., variation in density, solubility, and viscosity of solutions) (ANDRADE *et al.*, 1999). Cashew gum is a branched acidic heteropolysaccharide composed of a (1 $\rightarrow$ 3) β-D-galactose main chain with glucose side chains (PITOMBEIRA *et al.*, 2015). It is a low-viscosity polymer in aqueous solutions. Characterization of the monosaccharides present in the structure of the

Anacardium occidentale (cashew gum) exudate was performed by PAULA; HEATLEY; BUDD, (1998) and found to contain 73% galactose, 11% glucose, 5% arabinose, 4% rhamnose, 1% mannose, and 4.7% glucuronic acid, in addition to a polysaccharide-protein complex (RODRIGUES; PAULA; COSTA, 1993). The use of *A. occidentale* as a wall material for encapsulation has been previously studied (COMUNIAN *et al.*, 2018; DE SOUZA *et al.*, 2018). However, there are no reports on the use of galactomannan and cashew gum blends. In this study, the combination of these two polysaccharides (where cashew gum provided the coating functionality and galactomannan supplied a suitable viscosity) was evaluated to improve the encapsulation efficiency of *Rosmarinus officinalis* essential oil. In addition, the use of a cross-linking agent could be advantageous for microparticles. Cross-linking of biopolymers has already been shown to harden the microparticle wall, decrease porosity, and increase the retention of the core material (CHEN *et al.*, 2012; COMUNIAN *et al.*, 2016).

Thus, the aim of this study was to determine the optimal combination of galactomannan/cashew gum blends using a rotatable central composite design (RCCD) for the microencapsulation of rosemary essential oil with a detailed characterization of the processes and particles.

METHODS

Material

Rosemary (*Rosmarinus officinalis*) essential oil was purchased from Ferquima Ind. and Com. Ltda. Cashew gum was obtained from Embrapa Tropical Agroindustry. The *Caesalpinia pulcherrima* (galactomannan) seeds were handpicked from the metropolitan area of Fortaleza and identified in the Prisco Bezerra Herbarium (excerpt numbers 563667). All chemical reagents used were of analytical grade.

Cashew gum isolation

Polysaccharides from cashew gum were isolated according to a previously described method (TORQUATO *et al.*, 2004) with some modifications. The gum was ground in a mill and dissolved in water at a ratio of 1:4 (w/v) by stirring for 4 h without heating. Centrifugation was performed at 15.303 × g for 10 minutes, and the precipitate was discarded. Filtration and precipitation with commercial ethanol (96%) were subsequently performed with an ethanol-to-gum ratio of 3:1 (w/w) under refrigeration for 24 h. The precipitate was isolated and dried at 60°C. Finally, the gum was triturated and passed through a 212 µm sieve. The polysaccharide was kept in a hermetically sealed glass vial for later use.

Galactomannan extraction from *Caesalpinia pulcherrima* seed

Caesalpinia pulcherrima seeds were ground in a mill and separated into teguments, cotyledons, and endosperms. The endosperms were heated at 70°C in ethanol (96%), refluxed for 15 min, and left to swell at a proportion of 1:10 (endosperm to distilled water) for 24 h at 7°C. The intumescent endosperms were homogenized with distilled water until a viscous solution was obtained. The material was filtered through a nylon mesh, and the filtered solution was precipitated with ethanol (96%) in a ratio of 1:2 (v/v) solution/ethanol. Finally, the precipitate (galactomannan) was dehydrated with acetone, dried in a forced air circulation oven at 60°C for 24 h, and stored as dry powder for later use. The polysaccharide extraction yield from the seeds was expressed as the percentage of dry mass obtained after extraction in relation to the dry weight of the seeds.

Physicochemical characterization of matrix components

Determination of total nitrogen from polysaccharides

The total nitrogen content of polysaccharides (cashew gum and galactomannan) was determined after digestion of the samples with sulfuric acid according to a previously described method (BAETHGEN; ALLEY, 1989). The nitrogen content of the sample was calculated in relation to a standard curve in the presence of ammonium sulfate, and the crude protein content was calculated by multiplying the total nitrogen value by 6.25.

Ash content

The ash content of the polysaccharides was obtained from 2 g of each sample and placed in a muffle furnace (Thermo Scientific, Massachusetts, USA) at 550°C, where the organic material was completely incinerated. The ash content was calculated based on the relation between the residue weight and the initial sample weight, and the result was expressed as a percentage (HORWITZ, 2000).

Phenolic compounds

The total phenolic compound content was determined using the Folin–Ciocalteu method (LARRAURI; RUPÉREZ; SAURA-CALIXTO, 1997). The samples (1 g) were weighed and diluted in water. Then, 100 µL of the solution containing the sample was removed and mixed with 0.5 mL of Folin–Ciocalteu reagent (1:3), 1 mL of a 20% (w/v) sodium carbonate solution, and 1 mL distilled water. Absorbance was measured after 30 minutes using a

spectrophotometer at a wavelength of 700 nm. The results were expressed as milligrams of gallic acid (GA) times 100-L of the sample.

Experimental design

Response surface methodology was applied using a rotatable central composite design (RCCD) to determine the influence of two variables on the formation of microparticles by spray-drying. The two independent variables were related to the solids concentration: cashew gum (X1) and galactomannan (X2). The experimental design consisted of 11 treatments with three central points (Table 1).

Table 1. Rotatable central composite design used in rosemary essential oil microencapsulation trials based on 100 mL of distilled water.

Trials	Coded Variable		Real Variable		
	(X1)	(X2)	Cashew gum (g)	Galactomannan (g)	Rosemary essential oil (g)
1	-1	-1	18.29	0.32	2.5
2	1	-1	19.71	0.32	2.5
3	-1	1	18.29	0.88	2.5
4	1	1	19.71	0.88	2.5
5	-1.41	0	18	0.6	2.5
6	1.41	0	20	0.6	2.5
7	0	-1.41	19	0.2	2.5
8	0	1.41	19	1	2.5
9	0	0	19	0.6	2.5
10	0	0	19	0.6	2.5
11	0	0	19	0.6	2.5

Preparation of the emulsions

The emulsions were prepared according to RIBEIRO *et al.* (2015), with some modifications. Solutions were prepared by dissolving cashew gum and galactomannan in distilled water under magnetic stirring for 12 h at room temperature. After solubilization of the polysaccharides, each formulation was homogenized using an Ultra-Turrax homogenizer (IKA, T25 digital) at 20,000 rpm for 1 min, and then dispersed by ultrasound at 40x amplitude and 1 cycle for 30 s. Thereafter, 2.5% (w/v) rosemary essential oil and 6% (w/v) of the cross-linking agent TMF were added, and the pH was adjusted to 12 with 2 M NaOH. The emulsions were stirred for 30 minutes After this period, the solutions were neutralized to pH 7. Tween 80 and Span 80 emulsifiers were subsequently added to the solutions according to the hydrophilic-lipophilic balance (HLB value = 7.0) based on the concentration of essential oil used in the formulation. Finally, the emulsions were again homogenized using an Ultra-Turrax homogenizer (IKA, T25 digital) at 14,000 rpm for 5 minutes.

Characterization of emulsions

Emulsion viscosity

Rheological measurements of the samples were performed using an R/S-SST 2000 Brookfield-Searle cylindrical rotational rheometer. The measurements were performed at 25 °C, which was adjusted using a thermostatic bath coupled to the equipment. Rheological analyses were performed by varying the deformation rate from 0 to 500 s-1 (upward curve) for 1 min and recording 25 points for each curve. The flow curves were obtained from the shear stress (τ) versus shear rate (γ'), and the rheological parameters, pseudoplasticity index (*k*), and flow behavior index (*n*), were obtained by the power law using Equation 1. The apparent viscosity was analyzed using the graph as a function of the shear rate (*γ'*).

$$\tau = [(k * \gamma')]^n \quad (1)$$

Spray drying encapsulation

The emulsions were spray-dried using an atomizer (model MSD 1.0; Labmaq, Brazil) equipped with a 1.2 mm-diameter double-fluid spray nozzle. The inlet air temperature used was 170ºC, the flow of applied feed was 0.35 L/h and the airflow was 4.2 m^3/min. The dry powders obtained were stored under refrigeration in hermetic packaging.

Microparticle characterization

Water solubility

The solubility of the samples was determined according to a modified method described by CANO-CHAUCA *et al.* (2005). Overall, 0.25 g of each sample was weighed and 25 mL of distilled water was added to each portion. The solution was centrifuged (1.377 × g for 5 min at 25°C), and the supernatant was filtered. Twenty milliliters of the supernatant were added to the Petri dish. This material was dried in an air circulation oven at 105°C for 5 h. The solubility was calculated by the difference in mass (final mass–initial mass), and the results were expressed as a solubility percentage.

Particle size distribution

The particle size distribution was analyzed using a Malvern 3000 Zetasizer NanoZS laser light scattering instrument (Malvern Instruments, UK). For this evaluation, 0.50 g of sample powder was suspended in 50 mL of isopropyl alcohol (refractive index 1.39) at a ratio

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of 1:100 (w/v). The solution was maintained at room temperature for 24 h. The analysis was performed by withdrawing 1 mL of the solution from the upper section of the volumetric flask. The average diameter was determined by considering the average diameter of a sphere of the same area (surface area moment mean diameter, D [3,2].

Encapsulation efficiency

The encapsulation efficiency was calculated based on the total and surface oil contents using Equation 2.

$$EE\ \% = \frac{total\ oil - surface}{total\ oil} * 100 \quad (2)$$

The total oil content was determined from 10 g of encapsulated powder in 250 mL of distilled water using a Clevenger-type apparatus for 3 h (JAFARI *et al.*, 2008b). The determined volume was multiplied by the oil density (0.915 g/mL). Petroleum ether was used as the extraction solvent for surface oil measurement (JAFARI *et al.*, 2008b). The spray-dried powder (10 g) was dispersed in 25 mL of solvent with gentle shaking for 10 minutes at room temperature. The dispersion was filtered using a qualitative filter paper. The particles were washed three times with 5 mL of petroleum ether, and the filtered solution containing the extracted oil was transferred to a Petri dish to evaporate the organic solvent at room temperature. The surface oil mass was obtained from the difference in mass relative to the initial mass of the particles.

Retention efficiency

First, total oil was calculated by distilling 10 g of fresh encapsulated powder in 250 mL of distilled water for 3 h in a Clevenger apparatus. The oil volume was observed directly in the glass beaker and multiplied by its density. Oil retention was defined as the ratio of total oil extracted from the initial oil in the feed to the total solids (Equation 3).

$$Retention\ efficiency\ \% = \frac{oil\ content\ in\ microparticles}{initial\ oil\ content\ in\ emulsion} * 100 \quad (3)$$

Particle morphology

The particle morphology was evaluated using scanning electron microscopy (SEM). The samples were plated with gold in a sputtering-type apparatus and examined under a scanning electron microscope (MEV Zeiss DSM, model 940), operating at 15 kV with a magnification of 4000x.

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RESULTS AND DISCUSSIONS

Polysaccharides characterization

The physical and chemical characteristics of natural gums may be influenced by many factors, such as plant age, origin, climate, and biotic and abiotic stress (OKOYE; ONYEKWELI; KUNLE, 2012; GYEDU-AKOTO *et al.*, 2016). The polysaccharides isolated from gums presented a low content of protein, ash, and phenolic compounds (Table 2). The yield for cashew gum was superior to that of galactom almost three times.

Table 2. Analysis of protein, ash, phenolic compounds, and yield of galactomannan from *C. pulcherrima* and cashew gum from *A. occidentale L.*

Gums/Analysis	Protein (%)	Ash (%)	Phenolic Compounds (mg AG.100g^{-1})	Yield (%)
Galactomannan	0.65 ± 0.09	0.38 ± 0.03	-	25.00 ± 0.05
Cashew Gum Isolate	0.50 ± 0.07	0.74 ± 0.07	77.81 ± 1.58	70. 22 ± 3.67

Galactomannan extraction from *C. pulcherrima* resulted in a 25% (w/w) yield based on the seed mass. Cashew gum yields reported by other authors were approximately 80% (RODRIGUES; PAULA; COSTA, 1993) and 52% (OKOYE; ONYEKWELI; KUNLE, 2012), which were different from the yield obtained in this study (70%). These differences are associated with the aforementioned issues, such as plant origin and the methodologies used for polysaccharide isolation in each study. The protein content of *C. pulcherrima* galactomannan was 0.6%. This percentage is below the values reported in the literature for galactomannan obtained from the seeds of Mimosa scabrella, Stryphnodendron adstringens, and Schizolobium parahybae, where values ranged between 1.9–8.4% protein (SALVALAGGIO *et al.*, 2015).

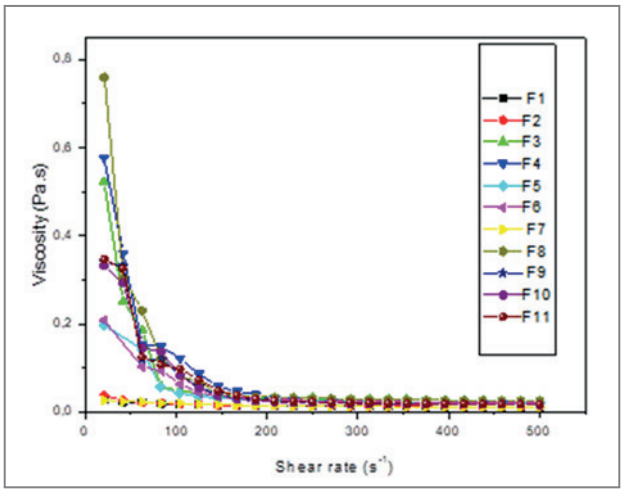
The protein content of CG was 0.50%, which was close to the value found for galactomannan. The ash content of isolated cashew gum was low compared to that found by COSTA, RODRIGUES, PAULA (1996) who reported ash contents in different stages of cashew gum purification that varied between 0.92–2.36%. The ash content of cashew gum was approximately 0.8%, which is similar to the value reported in this study (PORTO; CRISTIANINI, 2014). The content of phenolic compounds in isolated cashew gum was 77.81 mg AG per 100 g, possibly due to tannin traces present in the trunk bark of the plant from which the gum was extracted.

Characterization of emulsions

Emulsion viscosity

The emulsion viscosity can affect the encapsulation efficiency through the fluctuation and circulation of internal oil droplets during the drying process, thereby influencing the retention or loss of volatile compounds (JAFARI *et al.*, 2008a; BOTREL *et al.*, 2012). Higher viscosities were observed in the treatments that presented higher concentrations of galactomannan (Figure 1). The galactomannan from *C. pulcherrima* has high viscosity due to its chemical structure, since the mannose/galactose ratio influences its intrinsic viscosity and higher molecular weight. In addition to its chemical structure, intra- and intermolecular hydrogen bonding may also cause aggregation, increasing the viscosity of the emulsions (POLLARD *et al.*, 2010; MENDES *et al.*, 2017). The increase in viscosity can improve the retention of volatiles due to the rapid formation of a semipermeable membrane during the microencapsulation process (JAFARI *et al.*, 2008b).

Figure 1. Relationship between the apparent viscosity (μ) and shear rate (γ) of the rosemary essential oil emulsions, cashew gum, and galactomannan.



According to Table 3, the model was adjusted to the experimental data with R2 >0.98. The n values for the design emulsions were smaller (n <1), although they presented a behavior closer to that of pseudoplastic fluids. This is due to greater molecular alignment towards the formed flow, resulting in greater liquid fluidity and less friction (NSOFOR; OSUJI, 1997). A similar behavior was reported in an emulsion prepared with cashew gum and fish oil, presented as pseudoplastic fluids with n <1 (BOTREL *et al.*, 2012). In a study carried out by THOMBRE; GIDE, (2013) using galactomannan from *C. pulcherrima* at different concentrations, pseudoplastic behavior was clearly observed with 1% galactomannan, with reductions of pseudoplasticity occurring at 0.5% and 0.3% concentrations but still prevailing with n <1.

Table 3. Rheological parameters of design emulsions using the Power Law model.

Emulsions	Ascendent		
	K (Pa.s)	*n*	R2
F1	0.04 ± 0.002	0.78 ± 0.01	0.99
F2	0.04 ± 0.003	0.77 ± 0.01	0.99
F3	0.11 ± 0.02	0.75 ± 0.01	0.99
F4	0.12 ± 0.01	0.72 ± 0.01	0.99
F5	0.11 ± 0.01	0.69 ± 0.01	0.99
F6	0.14 ± 0.01	0.64 ± 0.01	0.98
F7	0.07 ± 0.04	0.68 ± 0.01	0.99
F8	0.25 ± 0.01	0.62 ± 0.01	0.99
F9	0.16 ± 0.01	0.62 ± 0.01	0.98
F10	0.14 ± 0.01	0.63 ± 0.01	0.99
F11	0.12 ±0.01	0.66 ± 0.01	0.99

Microparticle characterization

Solubility in water

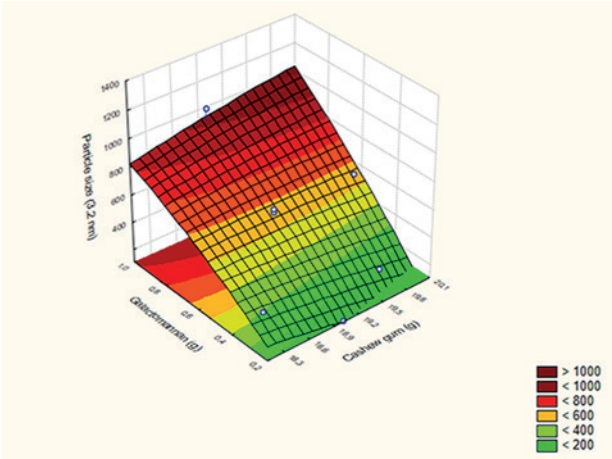
The food industry has understood that food ingredients or additives must exhibit good solubility or compatibility with the product. In general, consumers tend to reject a product if it contains clusters or lumps because of insoluble ingredients. Thus, all particles produced in food formulations need to be soluble or compatible, despite the hydrophobic nature of the core material and possible cross-linking, which could drastically reduce solubility. In this study, the solubility ranged between 79–82%, and there was no significant difference ($p > 0.05$) among the treatments.

Particle size distribution

The particle size in oil is a relevant factor for encapsulation because it is associated with oil stability and retention of particles, which can also affect the appearance, fluidity, and dispersibility of the product.

The particle size was significantly influenced by the matrix componentes, with D [3,2] values greater than 1000 nm obtained for microparticles with 19 g cashew gum and 1 g galactomannan (Figure 2). The production of larger particles in the presence of higher galactomannan concentrations may be related to the high viscosity of this polysaccharide. The particle size produced in the spray-drying process depends on the droplet size of the emulsion to be atomized (SHAMAEI *et al.*, 2017). Galactomannans are known to produce high-viscosity aqueous solutions even at low concentrations, which makes them commercially useful, mainly as thickening agents.

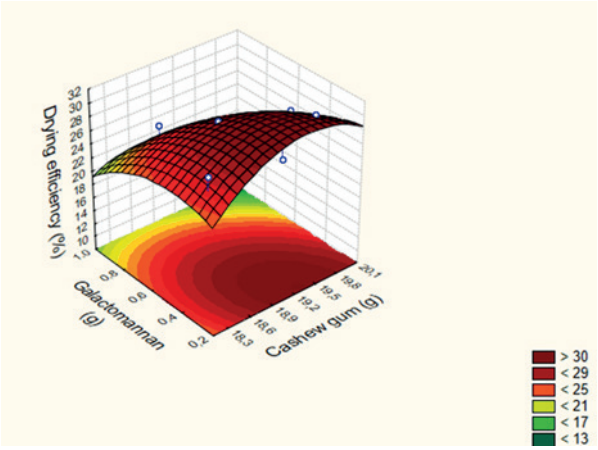
Figure 2. Response surface of particle size D [3,2] as powder obtained by spray drying using different amounts of galactomannan and cashew gum. Model = 566.60 - 10.48 (x1) - 16.34 (x1)2 + 582.78 (x2) - 47.67(x2) 2 + 116.44(x1). (x2) R2 = 0.98.



Efficiency of microparticle formation

Retention efficiency is defined as the amount of oil in the particle after encapsulation in relation to the initial amount of oil added to the emulsion. The best retention efficiency responses were obtained for 0.2–0.4 g of galactomannan and 18.9–19.8 g of cashew gum (Figure 3). Within this range, 0.4 g of galactomannan and 19.4 g of cashew gum were chosen, with the aim of using more galactomannan in the blend. In addition, galactomannan is a hemicellulose and a source of dietary fiber, which has valuable thickening, emulsifying, stabilizing, and film-forming capacity at low concentrations, which contributes to better retention of the nucleus material (THOMBRE; GIDE, 2013).

Figure 3. Response surface of drying efficiency obtained by spray drying with different amounts of galactomannan and cashew gum. Model = 28.72 + 0.60 (x1) 4.50 (x1)2 - 6.84 (x2) - 3.16 (x2)2 - 2.81(x1) · (x2) R2 = 0,90.



Encapsulation efficiency

One of the most important parameters for measuring the performance of the encapsulation method is the encapsulation efficiency, which is defined as the amount of oil effectively

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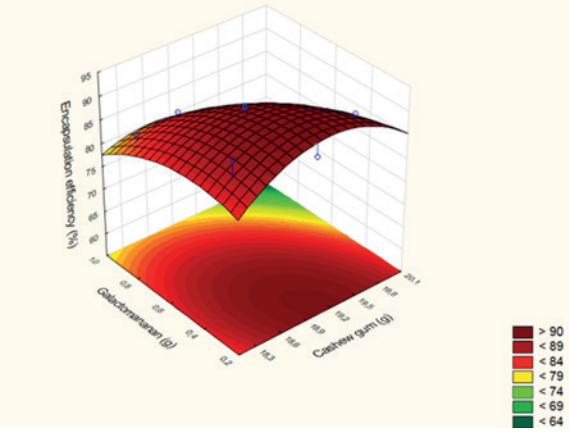
encapsulated (i.e., inside the particle). This is directly affected by the emulsion properties, solid content, viscosity, and droplet size (JAFARI *et al.*, 2008a). Using this parameter, it is possible to evaluate the presence of oil on the surface, which is prone to oxidation; thereby, deteriorating the powder quality (FERNANDES *et al.*, 2014). Moreover, surface oil may decrease powder wettability and render it less soluble in water. The encapsulation efficiency varied between 74.5–91.33%, which was significantly influenced by the blend concentration of the two matrix components. In view of the results, the best combinations were found to be approximately 0.4% (w/v) galactomannan and 19.4% (w/v) cashew gum (Figure 4).

Figure 4. Response surface of encapsulation efficiency obtained by spray drying with different amounts of galactomannan and cashew gum. Model = 89.66 - 2.15 (x1) - 7.74 (x1)2 - 9.30 (x2) - 4.15(x2)2 - 3.86 (x1) (x2) R2 = 0.87.



Fonte: Author.

CONCLUSION

Rosmarinus officinalis L. (rosemary) essential oil has antibacterial and antioxidant properties. It is used mainly in the food industry and is associated with flavors that are also present in the oil. The use of biocompatible cashew gum and galactomannan gum may constitute an alternative wall material for rosemary essential oil encapsulation using the spray drying technique.

In this study, we have combined the high viscosity of galactomannan with the coating functionality of cashew gum. A suitable formulation was determined using response surface methodology. The increase in galactomannan content in the blend significantly affected the viscosity before spray drying. These emulsion characteristics influenced the resulting oil particles, which displayed a higher retention and encapsulation efficiency of rosemary oil using 0.4% (w/v) galactomannan and 19.4% (w/v) cashew gum.

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