

OPTIMIZATION OF EUGENOL-LOADED MICROCAPSULES OBTAINED BY SPRAY DRYING USING A SIMPLEX-CENTROID MIXTURE DESIGN**Paulo Rodrigo Stival Bittencourt^{a,*}, Ruth dos Santos da Veiga^{b,#}, Rosana Aparecida da Silva-Buzanello^{b,#}, Fernando Reinoldo Scremin^a, Tania Aparecida Becker-Algeri^{b,c,#,&}, Marinês Paula Corso^{b,#}, Éder Lisandro de Moraes Flores^a and Cristiane Canan^{b,#}**^aDepartamento de Química, Universidade Tecnológica Federal do Paraná, 85884-000 Medianeira – PR, Brasil^bUniversidade Tecnológica Federal do Paraná, 85884-000 Medianeira – PR, Brasil^cUniversidade do Rio Grande, 96203-900 Rio Grande – RS, Brasil

Recebido em 15/02/2021; aceito em 22/07/2021; publicado na web em 20/08/2021

Eugenol antioxidant and antibacterial properties have suggested its use as a natural preservative for food matrices. Eugenol application is compromised due to its high volatility and sensibility to external agents, such as light, heat, oxygen, and moisture. Thus, microencapsulation is viable to increase the eugenol stability. Results have showed that carrageenan combined with rice protein and bovine albumin allow high-quality eugenol microcapsules. These materials show excellent properties, favoring the microcapsules use in food matrices. This study aimed to optimize microcapsules formulation by spray drying, using a simplex-centroid mixture design. Blends of low-lactose whey protein (WP) and/or rice bran protein (RBP) and/or bovine serum albumin (BSA) with carrageenan were used as wall materials. Blends influenced the eugenol retention, microencapsulation efficiency, and size. Pure WP was not efficient for eugenol encapsulation. The highest eugenol retention values, 89.7% and 86.5% were obtained by combining carrageenan with RBP or BSA. RBP and BSA binary interaction with carrageenan showed the highest encapsulation value (80.5%). Scanning electron microscopy, infrared spectroscopy, and thermogravimetry confirmed eugenol inside the microcapsules.

Keywords: encapsulation efficiency; bovine serum albumin; low-lactose whey protein; rice bran protein.

INTRODUCTION

Eugenol (C₁₀H₁₂O₂; 4-allyl-2-methoxyphenol) is a phenolic compound extracted from spices and herbs, such as clove, basil, nutmeg, and cinnamon. The use of eugenol in food matrices has been suggested as a replacement for synthetic additives due to its potential antioxidant and antimicrobial characteristics.¹⁻³ However, eugenol is a hydrophobic compound that presents high volatility and sensitivity toward external agents, such as light, heat, oxygen and moisture, hampering its large-scale use in the food industry.⁴ Thus, microencapsulation is a viable alternative for greater stability for eugenol application.

The spray drying method is commonly used for bioactive compounds microencapsulation, such as flavor, lipids, and carotenoids.⁵ The technique comprises of preparing a suspension or emulsion of materials (encapsulant and encapsulated) followed by drying, under controlled dryer temperature and in feed flow rate. Rapid solvent evaporation enables the stability of microcapsules produced, with water as one of the most used solvents in this method.^{6,7}

Encapsulation efficiency and microcapsule stability may be influenced by the material used in microencapsulation.⁸⁻¹⁰ Despite the high variability of polymers available for use as wall material, biocompatible and biodegradable materials have been suggested for microencapsulation. Carbohydrates, gums, lipids, and proteins are a few examples of wall materials most commonly used in microencapsulation.^{11,12}

Carrageenan is a sulfated anionic polysaccharide extracted from red seaweed (class *Rhodophyceae*).¹³ The κ-carrageenan is characterized by a linear chain of β(1→3)-D-galactose sulfate and

α(1→4)-3,6-anhydro-D-galactose units chain that may be solubilized in water under heating (above 60 °C). After cooling, the galactose chains form double helices producing a three-dimensional network that characterizes the gel formation presented by carrageenan.¹⁴ K-carrageenan gel formation has been used in bioactive compounds microencapsulation by spray drying.^{14,15}

Proteins amphiphilic nature is responsible for reducing the oil-water surface interfacial tension,¹⁶ which favors their use in hydrophobic compounds microencapsulation. Bovine serum albumin (BSA) represents 42% of bovine blood proteins¹⁷ and shows functional technological properties allowing its application in several food matrices. Whey protein presents a globular structure and is soluble at a wide pH range. The diverse protein group that constitutes whey protein characterizes its functionality for a wide range of food applications.¹⁸

The properties of gelling and emulsification of whey proteins have been used by their application in food products. The presence of β-lactoglobulin as the main protein component of whey protein, which confers its gelling property.¹⁹ Therefore, the physicochemical properties of the whey protein suggest their use in other applications, such as the wall material in the microencapsulation. Whey protein has been reported in volatile compounds microencapsulation by spray drying.^{12,20,21} As the high lactose content in the wall materials was related to accelerating the flavor release and relative humidity,²² the application of low-lactose whey protein is suggesting in microencapsulation.

Rice bran proteins have some practical properties such as high foaming capacity and stability over time, as well as emulsifying capacity, similar to casein.²³ These proteins are obtained from rice bran, a protein-source-potential, low-cost, and high-quality by-product, containing 75% glutenine, 15% globulin, 6% albumin, and 3% prolamins.^{24,25} Rice bran proteins characteristics suggest their use as an alternative wall material in microencapsulation.

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Protein-carbohydrate blends used as wall materials may be an alternative for obtaining stable microcapsules.^{2,26} Thus, the simplex-centroid mixture design, considered an efficient methodology to determine optimum components levels and main reagents of formulation development, was used to evaluate the components blends effect.²⁷

Therefore, this study aimed to optimize the microcapsules formulation loaded with eugenol, with blends of low-lactose whey protein, rice bran protein, and bovine serum albumin, and with carrageenan as wall material, by spray drying. This study was based on a simplex-centroid mixture design, including the obtained microcapsules characterization.

EXPERIMENTAL

Eugenol (Sigma-Aldrich, 98%), ethanol P.A. (Neon, 99.5%), and Tween-80 (Synth) were used as an organic phase in the blend preparation. Carrageenan (C1013, Sigma-Aldrich, predominantly κ and lower amounts of λ carrageenan), extracted rice bran protein (50% protein dry basis),²⁸ bovine serum albumin (Sigma-Aldrich, 96%), and low-lactose whey protein (2.89% lactose, and 37% protein, produced according to Pacheco *et al.*)²⁹ were used as wall materials. In the extraction of protein from rice bran, the defatted rice bran suspension in ultrapure water was adjusted to pH 10 (NaOH 2 mol L⁻¹). The suspension was stirred at 80 rpm for 5 h at 45 °C. The pH was corrected to pH 4.5 using HCl solution (3 mol L⁻¹), and then the suspension was centrifuged for 15 min (25 °C). The precipitate containing the rice protein was washed and neutralized until reaching pH 7 and spray-dried to obtain rice bran protein.²⁵

Simplex-centroid mixture design

The effects of using carrageenan combined with low-lactose whey protein (X_1), rice bran protein (X_2), and bovine serum albumin (X_3) for eugenol microencapsulation were evaluated by a simplex-centroid mixture design. Carrageenan and protein concentrations were fixed at 0.5 and 3.0% (w/v), respectively, in all assays, determined by preliminary tests.

Microcapsules preparation

The eugenol-loaded microcapsules were obtained from an organic and aqueous phases suspension followed by drying. Eugenol (0.80 g) and Tween-80 (0.12 g) were dissolved in ethanol (10 mL) for 2 min, resulting in the organic phase. For the aqueous phase, carrageenan (0.5%, w/v) was suspended in 200 mL distilled water at 60 °C and stirred for 15 min. The separated single proteins were suspended in distilled water by mechanical stirring for 10 min, as per ratios presented in Table 1, resulting in 200 mL protein suspension (3%, w/v). The organic phase was poured into the aqueous phase and vigorously stirred for 5 min. The suspension obtained was then homogenized using the Fisaton™/713D homogenizer at 1500 rpm for 15 min.

The suspension spray drying was done using a mini spray dryer (Labmaq™ -MSD 1.0), with a 500 mm x 150 mm drying chamber, double fluid type nozzle atomizer with a 1.2 mm diameter opening. The encapsulating compositions were fed by a peristaltic pump was used at a 0.6 L h⁻¹ flow rate, 150 °C inlet air temperature, 110 °C outlet temperature, and a 35 N m³ h⁻¹ (normal cubic meter *per* hour) drying air flow rate. The atomizing air flow was kept at 35 L min⁻¹ and 300 kPa.

Eugenol retention ratio determination

The total eugenol concentration ([eugenol]_{total}) in the microcapsules obtained (encapsulated and non-encapsulated – adhered to the outer part of the microcapsule surface) was obtained using 10 mg microcapsules. The samples were solubilized in 10 mL ethanol/water 1:1 (v/v) and homogenized in ultrasound (Elmasonic™, P60H), at 37 kHz frequency and 37% amplitude. The samples were centrifuged (Hettich Zentrifugen™, Rotina 420) at 9000 rpm for 15 min. The supernatant was filtered (Millipore 0.45 μ m), and 1 mL was quantified. Eugenol quantification was performed in an ultra-high performance liquid chromatographer coupled to a UV-Vis detector (UHPLC-UVD170U, Dionex Corporation™, UltiMate 3000). Methanol/water 85:15 (v/v) ratio was used as mobile phase for eugenol simultaneous separation in the isocratic elution method. Reverse phase C18 Acclaim PA2 C18 column 5 μ m (250 x 4.6 mm) was used with 1 mL min⁻¹ mobile phase flow and column temperature at 25 °C. Eugenol detection was conducted at 280 nm with a 20 μ L sample injection volume. The chromatographic test lasted 10 min and eugenol retention time was 5.7 min. Eugenol quantification was performed based on a calibration curve of eugenol solutions in methanol at 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 g L⁻¹ concentrations.

Eugenol retention ratio (ER) was determined according to Equation 1.

$$ER(\%) = \frac{[eugenol]_{total}}{[eugenol]_{initial}} \times 100 \quad (1)$$

Eugenol encapsulation efficiency determination

Non-encapsulated eugenol was removed by washing 10 mg microcapsules samples with 10 mL ethanol. Microcapsules samples were then dried at 50 °C in a circulation oven. The dry samples were solubilized in an ethanol/distilled water 1:1 (v/v) ratio solution (10 mL) and the encapsulated eugenol content was determined by UHPLC as per the described previously methodology. Eugenol encapsulation efficiency (EE) was calculated using Equation 2.

$$EE(\%) = \frac{[eugenol]_{encapsulated}}{[eugenol]_{total}} \times 100 \quad (2)$$

Microcapsules morphology and average size

Microcapsules morphology and average size were investigated by scanning electron microscopy (SEM, Hitachi, TM 3000) at 15 kV. The samples were deposited over adhesive tape and gold-coated with a vacuum sputtering coater. The average microcapsules size was determined by measuring 120 particles from each formulation using SEM images.³⁰

Fourier Transform Infrared (FTIR) Analysis

Pure eugenol, eugenol-loaded microcapsules from assays 3 and 6, and microcapsules added with 50% pure eugenol (w/w) (MC + 50% eugenol) were analyzed by FTIR spectroscopy (Frontier PerkinElmer™ with attenuated total reflectance mode). The spectra were recorded at wavenumber ranging from 4000 to 700 cm⁻¹, at room temperature, using 32 accumulated scans and a 4 cm⁻¹ resolution.

Thermogravimetric analyses

Thermogravimetric analyses (TG) were done by simultaneous

thermo-analyzer STA6000 (PerkinElmer™). About 10 mg samples were placed on a platinum crucible and heated from 50 to 600 °C at a 10 °C min⁻¹ heating rate, in a dynamic nitrogen atmosphere with a 20 mL min⁻¹ flow rate.

Statistical analyses

Scheffe's canonical model, determined by Equation 3, was used to adjust eugenol retention and encapsulation efficiency experimental data. Linear, quadratic, and cubic models were tested to obtain the respective regression coefficients, using regression and analysis of variance (ANOVA).

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (3)$$

where Y is the response as a function of independent variables X_1 , X_2 , and X_3 in their codified levels; β_1 , β_2 , and β_3 are the regression coefficients of the primary interactions; β_{12} , β_{13} , and β_{23} are the regression coefficients of binary interactions; and β_{123} is the regression coefficient of ternary interaction.

Response surfaces were determined using mathematic models ($p \leq 0.05$) to establish the optimal microencapsulation process conditions based on eugenol retention and encapsulation efficiency. Global desirability function was determined using the Response Desirability Profiling procedure. Statistical analyses were conducted using the Statistica 7.0 software (Statsoft Inc., Tulsa, OK, USA, 2004).

RESULTS AND DISCUSSION

Eugenol-loaded microcapsules optimization

Table 1 shows the simplex-centroid mixture design responses for eugenol retention (Y_1), encapsulation efficiency (Y_2), and average microcapsules size (Y_3). Eugenol retention results ranged from $37.9 \pm 0.1\%$ to $89.7 \pm 1.3\%$ and for encapsulation efficiency from $0.0 \pm 0.0\%$ to $80.5 \pm 8.7\%$, showing they were significantly influenced by wall materials contents.

The average microcapsules size ranged from $3.4 \pm 2.3 \mu\text{m}$ to $4.6 \pm 2.1 \mu\text{m}$. Similar results were reported by Edris *et al.*³¹ ($4.37 \mu\text{m}$) and Chatterjee & Bhattacharjee¹ (1 to 15 μm), who respectively studied the *Nigella sativa* L. (black cumin) essential oil and eugenol-rich clove extracts microencapsulation, in gum Arabic

and maltodextrin mixture. Locali Pereira, Gonçalves Cattelan & Nicoletti³² when microencapsulated pink pepper essential oil in pectin, soy protein isolate, and maltodextrin by spray drying, obtained values between 4.19 and 5.92 μm , similar to those found in this study. These microcapsules size variability is typical from the spray drying process.

Eugenol retention (Y_1), encapsulation efficiency (Y_2), average microcapsules size (Y_3), and their respective regression coefficients and mathematical variance analysis models were obtained (Table 2). The mathematical models for Y_1 and Y_2 showed significant linear, quadratic, and cubic effects at a 95% significance level, and due to the Y_3 model, the results observed for the cubic model did not show any significant effects ($p > 0.05$). Cubic models for a three-component-mixture have linear terms related to the additive model, quadratic terms representing binary interactions, and cubic terms that are related to the ternary interaction effect. The design generally performed to determine the model's coefficient values is named simplex-centroid or special cubic model. Therefore, the linear, quadratic, and cubic terms are usual for simplex-centroid mixture design and thus, have been determined.³³

Additionally, the coefficients of determination (R^2) were 0.993, 0.995, and 0.830 for Y_1 , Y_2 , and Y_3 , respectively, and no mathematical model showed any significant lack of fit ($p > 0.05$). However, only Y_1 and Y_2 models were significant ($p \leq 0.05$) to predict the evaluated responses. Then, the response surface, using a simplex centroid mixture design, was applied for valid models (Y_1 and Y_2). Contours plots are shown in Figure 1 (a, b).

Combined carrageenan and rice bran protein (X_2), or bovine serum albumin (X_3), as microcapsules wall materials resulted in the highest eugenol retention values (Y_1), 89.7 and 86.5%, respectively. Eugenol retention determination may be used as an indirect suspension stability measuring. Proteins have volatile compounds entrapping properties by either Van der Waals interactions, hydrogen interaction, or electrostatic interaction. In an aqueous system, interactions between volatile compounds non-polar portions and hydrophobic protein occur.^{34,35} Thus, obtaining the encapsulating suspension favours hydrophobic interactions between eugenol proteins, minimizing the eugenol operational losses under these experimental conditions.

Surface hydrophobicity is linked with emulsifying properties, hence hydrophobic interactions, once hydrophobic interactions have an important role in this protein functional property. Wang *et al.*³⁶ described that rice bran protein concentrate had a lower surface hydrophobicity (12.2 ± 2.2) than bovine serum albumin (86.4 ± 1.2) and thus a lower emulsifying property. The low rice bran protein

Table 1. Simplex-centroid mixture design for eugenol retention (%), encapsulation efficiency (%), average size (μm) responses, and eugenol-encapsulated content (ppm) of eugenol-loaded microcapsules (n = 3)

| Assays | Proteins proportion used as encapsulating agents ¹ | | | Function response ² | | | Eugenol-encapsulated ⁶ (ppm) |
|--------|---|--------------------|--------------------|--------------------------------|----------------|---------------|---|
| | X_1 ($x_1/\%$) | X_2 ($x_2/\%$) | X_3 ($x_3/\%$) | Y_1^3 | Y_2^4 | Y_3^5 | |
| 1 | 1.00 (3) | 0.00 (0) | 0.00 (0) | 58.1 ± 7.7 | 0.0 ± 0.0 | 3.4 ± 2.3 | $0.0^d \pm 0.0$ |
| 2 | 0.00 (0) | 1.00 (3) | 0.00 (0) | 89.7 ± 1.3 | 27.6 ± 6.5 | 3.5 ± 1.9 | $40.4^b \pm 9.5$ |
| 3 | 0.00 (0) | 0.00 (0) | 1.00 (3) | 86.5 ± 0.8 | 45.1 ± 3.5 | 4.6 ± 2.1 | $63.8^a \pm 5.0$ |
| 4 | 0.50 (1.5) | 0.50 (1.5) | 0.00 (0) | 37.9 ± 0.1 | 47.8 ± 8.7 | 4.4 ± 2.5 | $29.4^{bc} \pm 5.4$ |
| 5 | 0.50 (1.5) | 0.00 (0) | 0.50 (1.5) | 40.2 ± 10.4 | 47.6 ± 4.8 | 3.8 ± 1.7 | $31.3^{bc} \pm 3.2$ |
| 6 | 0.00 (0) | 0.50 (1.5) | 0.50 (1.5) | 52.4 ± 4.2 | 80.5 ± 8.7 | 3.7 ± 2.0 | $69.0^a \pm 7.5$ |
| 7 | 0.33 (1) | 0.33 (1) | 0.33 (1) | 68.3 ± 0.0 | 11.7 ± 2.2 | 3.8 ± 2.1 | $12.9^{cd} \pm 2.4$ |
| 8 | 0.33 (1) | 0.33 (1) | 0.33 (1) | 66.9 ± 1.4 | 18.3 ± 1.1 | 4.2 ± 1.9 | $19.9^c \pm 1.2$ |
| 9 | 0.33 (1) | 0.33 (1) | 0.33 (1) | 62.4 ± 4.0 | 17.5 ± 0.0 | * | $16.9^{cd} \pm 0.0$ |

¹Carrageenan and proteins concentrations were kept fixed in all experimental conditions —0.5% (w/v) and 3% (w/v), respectively. These concentrations correspond to the final experimental design proportion (suspension). X_1 , X_2 , and X_3 = codified values for low-lactose whey protein, rice bran protein, and bovine serum albumin proteins proportions used in mixtures, respectively. x_1 (%), x_2 (%), and x_3 (%) = real concentration values (w/v) of respective proteins added to mixture. ²Mean \pm standard deviation. ³ Y_1 = eugenol retention (%), ⁴ Y_2 = encapsulation efficiency (%), ⁵ Y_3 = average size (μm), ⁶Eugenol-encapsulated = quantified by UHPLC (n = 3). Different letters in the same column indicate statistical differences evaluated by the Tukey test ($p \leq 0.05$). *Not determined.

Table 2. Regression coefficients and analysis of variance of mathematical models adjusted to response function

| Coefficient | Response function ² | | |
|------------------|--------------------------------|----------------|----------------|
| | Y ₁ | Y ₂ | Y ₃ |
| Linear | | | |
| β ₁ | 58.10* | <-0.01 | 3.34* |
| β ₂ | 89.70* | 27.60* | 3.38* |
| β ₃ | 86.50* | 45.10* | 4.39* |
| Quadratic | | | |
| β ₁₂ | -144.00* | 136.00* | 3.74* |
| β ₁₃ | -128.40* | 100.20* | - |
| β ₂₃ | -142.80* | 176.60* | - |
| Cubic | | | |
| β ₁₂₃ | 915.30* | -1465.20* | - |
| R ² | 0.993 | 0.995 | 0.830 |
| p value | 0.0218* | 0.0159* | 0.0509 |

X₁, X₂, and X₃ = codified values for low-lactose whey protein, rice bran protein, and bovine serum albumin proportions applied in mixtures, respectively.

²Y₁ = eugenol retention (%), ³Y₂ = encapsulation efficiency (%), ⁴Y₃ = average size (μm). *Significant at a 95% confidence level.

hydrophobicity does not facilitate proteins and oils interaction, decreasing oil's absorption capacity.³⁷ Such considerations justify the lower Y₂ value (27.6 ± 6.5%) when using 100% rice bran protein (X₂). In addition to the properties of the proteins studied, it is important to highlight the interaction between proteins and carrageenan. Protein-polysaccharide interaction is provided by covalent bonds or physically charge distribution. The structure and stabilizing properties of the mixed protein-polysaccharide layer is depending of biopolymers adsorption on the interface, as well as, affected by pH, ionic strength, and biopolymer charge distribution.³⁸

Moreover, rice bran protein (X₂) and bovine serum albumin (X₃) binary interaction used in assay 6 with carrageenan, showed the highest encapsulation efficiency value (Y₂) (80.5 ± 8.7%) and the highest coefficient value (Y₁) (52.4%). Fabian, Huynh, & Ju,³⁹ when studying the rice bran proteins interaction with polysaccharides alginate and carrageenan to form complex precipitates reported that rice bran proteins may associate with polysaccharides since the obtained precipitate had foaming and emulsifying abilities. Thus, X₂ and X₃ combined with carrageenan favored protein-eugenol and protein-carrageenan interactions, contributing to encapsulation during the spray drying process.

Low-lactose whey protein (X₁) was not efficient for eugenol encapsulation. The X₁ and carrageenan combination did not allow eugenol loading (Y₂ = 0%) and encapsulation was not efficient when X₁ was combined with X₂ or X₃. Rosenberg & Sheu⁴⁰ reported that lactose-free whey protein did not contribute to the volatile compounds retention during the microencapsulation process. During spray drying, the presence of lactose in its amorphous state forms a continuous glass phase, in which protein chains are dispersed. Thus, the low-lactose whey protein prevents the film formation during drying and hinders eugenol entrapping.

The ternary protein mixture negatively affected the encapsulation efficiency (Y₂) and such effect may be observed by the negative value obtained for the β₁₂₃ coefficient value, as shown in Table 2. Therefore, the X₂ and X₃ proportions in the experimental conditions may not have been efficient to improve the film properties. Furthermore, due to X₂ and X₃ results in the binary interactions, the low-lactose whey protein (X₁) presence in the ternary interaction may have hampered the film formation process.

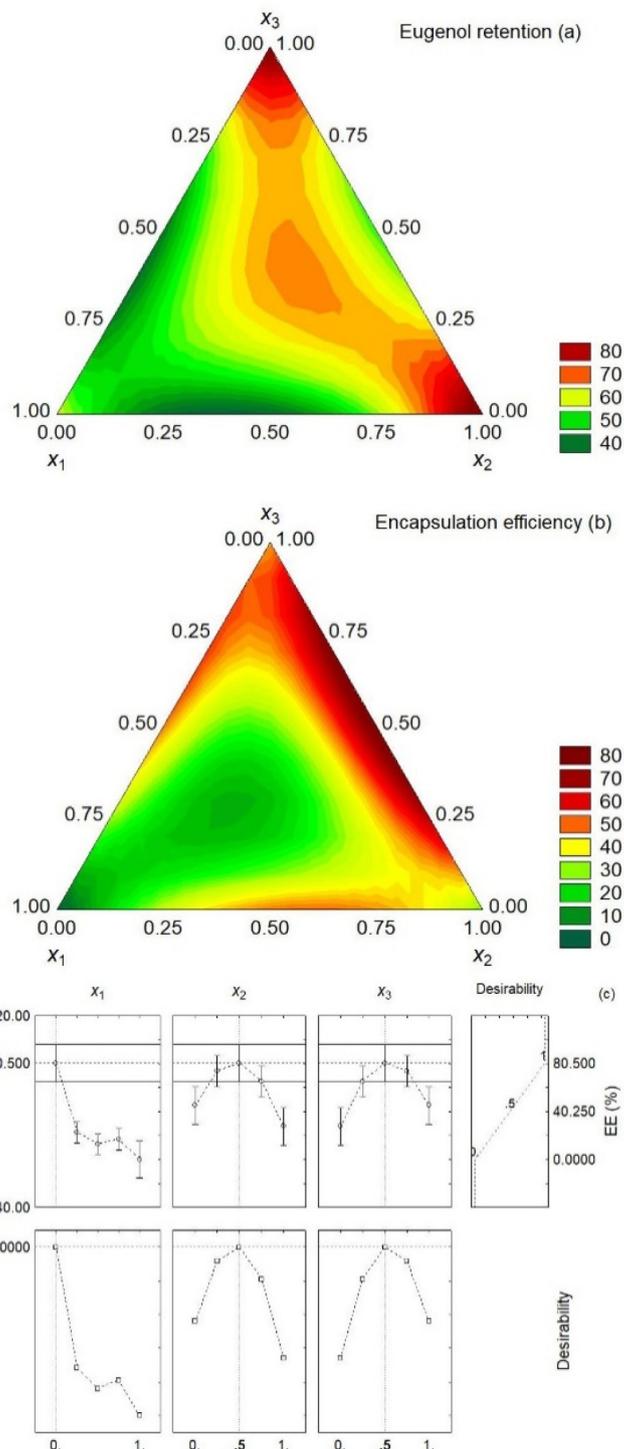


Figure 1. Simplex centroid mixture design for (a) eugenol retention, (b) encapsulation efficiency responses, and (c) optimal proportions for eugenol encapsulation efficiency (EE %) using the desirability parameters from low-lactose whey protein (X₁), rice bran protein (X₂), and bovine serum albumin (X₃) mixtures in eugenol microencapsulation (n = 3)

An optimal region prediction for the eugenol-loaded microcapsules formulation was possible from the estimated Y₁ and Y₂ responses analyses, by observing the response surface presented in Figures 1a and 1b, as well the optimal parameters. However, as the encapsulation efficiency (Y₂) is a direct determination of the eugenol amount encapsulated in the microcapsules, such response was the only one used to determine the desirability parameters (Figure 1c). For

the microcapsules produced, the optimal encapsulation efficiency (Figure 1c) occurred when $X_1 = 0$, $X_2 = 0.5$ and $X_3 = 0.5$ from assay 6 (Table 1) with Y_2 (80.5 %). Considering the optimum encapsulation efficiency model, the predicted value was 80.3%. A validation assay was performed in duplicate using the optimum condition variables, and the encapsulation efficiency value obtained was 79.9 ± 7.9 , with a relative deviation of 0.50%, compared to predict value. No statistically significant difference ($p > 0.05$) was found between the observed and the predicted responses by the encapsulation efficiency model; therefore, the model was validated.

Assay 6, which considers binary X_2 (rice bran protein) and X_3 (bovine serum albumin) interaction with carrageenan, showed the highest value for eugenol-encapsulated content (69.0 ± 7.5 ppm), and it was statistically similar ($p > 0.05$) to assay 3 when X_3 primary interaction with carrageenan was 63.8 ± 5.0 ppm. Assay 2, which considers X_2 primary interaction with carrageenan, had a 40.4 ± 9.5 ppm content value. Despite the high suspension stability obtained from assays 2 and 3, these proteins were not able to effectively bind eugenol when used with carrageenan alone, which reinforces the effect of the X_2 and X_3 combined use in the experimental conditions.

Microcapsules Characterization

Eugenol-loaded microcapsules morphology

Figure 2 presents the eugenol-loaded microcapsules SEM images. A spherical morphology may be observed in the microcapsules' SEM images obtained using the X_2 (rice bran protein) and X_3 (bovine serum albumin) mixture with carrageenan (Figures 2e and 2f). This microcapsule outer surface characteristic indicates the existence of solid walls ensuring low permeability

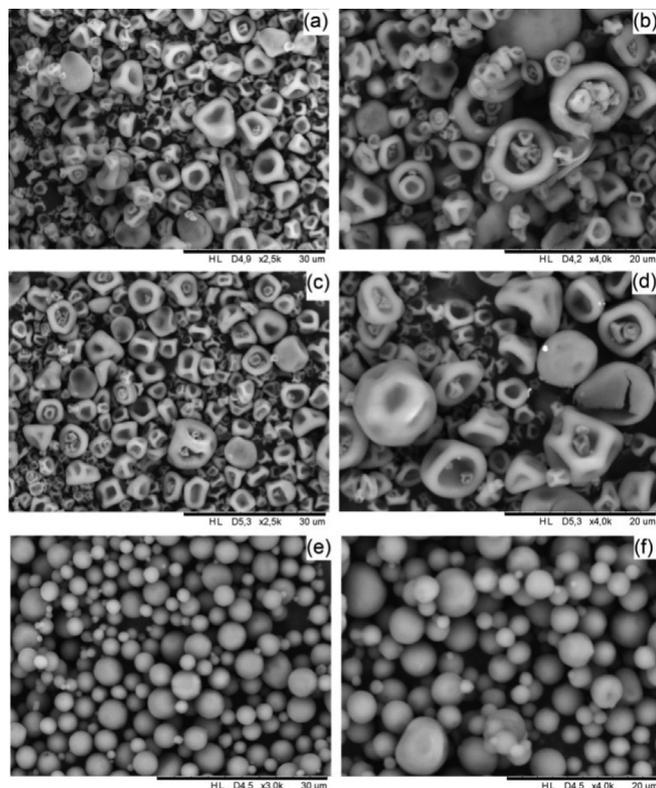


Figure 2. Eugenol-loaded microcapsules SEM images of carrageenan combined with rice bran protein (a, b); bovine serum albumin (c, d); and rice bran protein and bovine serum albumin binary mixture (e, f) produced by spray-drying. 2500x (left) and 4000x (right) magnification

to gases, better protection, and essential eugenol retention.²⁷ This morphologic characteristic corroborates with the encapsulation efficiency results shown, indicating better volatile compounds preservation in the essential oil, which implies lower permeability to gases that may cause oxidation.³²

However, the X_1 (low-lactose whey protein) and X_3 (bovine serum albumin) mixture and the isolated use of X_2 (rice bran protein) or X_3 (bovine serum albumin) with carrageenan resulted in microcapsules with deformations and surface roughness, suggesting that these mixtures used as wall materials were not suitable for obtaining microcapsules with good elasticity during the drying process. Some microcapsules have also presented fissures that characterized collapse (Figure 2b and 2d). Similar results were reported by Edris *et al.*³¹ when they obtained essential oil microcapsules by spray drying. The microcapsules roughness characteristic may be due to instantaneous sprayed oil resin emulsion droplets shrinkage during the early spray drying stages.

Fourier Transform Infrared (FTIR-ATR) spectroscopy

FTIR spectroscopy was used to investigate the microcapsules encapsulation efficiency. Bioactive compounds characteristic IR absorption bands in microcapsules FTIR spectra suggest their presence on the microcapsules surface, indicating low encapsulation efficiency.⁴¹

FTIR spectra were obtained for the assays that presented the highest encapsulated eugenol content (assays 3 and 6) and for the microcapsules added with pure eugenol (50% w/w), as shown in Figure 3.

For the eugenol FTIR spectrum, absorption bands at 1510 cm^{-1} ($\nu(\text{C}=\text{C}$ aromatic ring), 1265 cm^{-1} ($\nu(\text{C}-\text{O})$), 1230 cm^{-1} ($\nu(\text{C}-\text{O}-\text{C})$), and 1032 cm^{-1} ($\nu(\text{C}-\text{O}$ aromatic ring) were observed. Such bands were also reported by Chowdhry, Ryall, Dines & Mendham,⁴² and by Piletti *et al.*⁴³

Microcapsules samples FTIR spectra showed pronounced bands characteristic of wall material used in microencapsulation. The amide II band present in rice bran protein and bovine serum albumin was observed at 1535 cm^{-1} ($\nu(\text{N}-\text{H}$ bending / $\text{C}-\text{N}$ stretching).⁴⁴ It was noted a band at 1065 cm^{-1} attributed to carrageenan glycosidic bond.^{45,46} In addition to the high intensity band characteristic of wall material, shoulders were observed in FTIR spectrum of microcapsules added with pure eugenol (MC + 50%), indicating the presence of this compound in the system (Figure 3a). The shoulders observed at 1517 cm^{-1} and 1039 cm^{-1} , corresponding to eugenol, were also identified in MC 3 and MC 6 samples, however, with a lower intensity than that observed in the MC + 50% spectrum.

To facilitate observation of eugenol characteristic bands in microcapsules' FTIR spectra, bands deconvolution from 1560 to 1500 cm^{-1} , and from 1090 to 1020 cm^{-1} was performed for MC 6 (Figure 3b). A coefficient of determination (R^2) higher than 0.995 was obtained for both deconvoluted bands, showing optimal data fitting. The shoulders corresponding to eugenol (Figure 3b) were observed, confirming its presence in the microcapsules' wall.

Thermogravimetric analyses

Thermogravimetric analyses (TG) and their derivatives (DTG) for carrageenan (CA), rice bran protein (RBP), bovine serum albumin (BSA), and eugenol, as well as eugenol-loaded microcapsules assays 3 (MC 3) and 6 (MC 6), are shown in Figure 4. According to TG and DTG analyses, eugenol presented a single thermal loss of weight event at $246\text{ }^\circ\text{C}$, which may be characterized as eugenol evaporation. Nuchuchua *et al.*⁴⁷ reported a similar value ($241\text{ }^\circ\text{C}$) for the same event. Also, TG analysis showed a microcapsules' loss of weight, with the increase of temperature similar to the one for wall

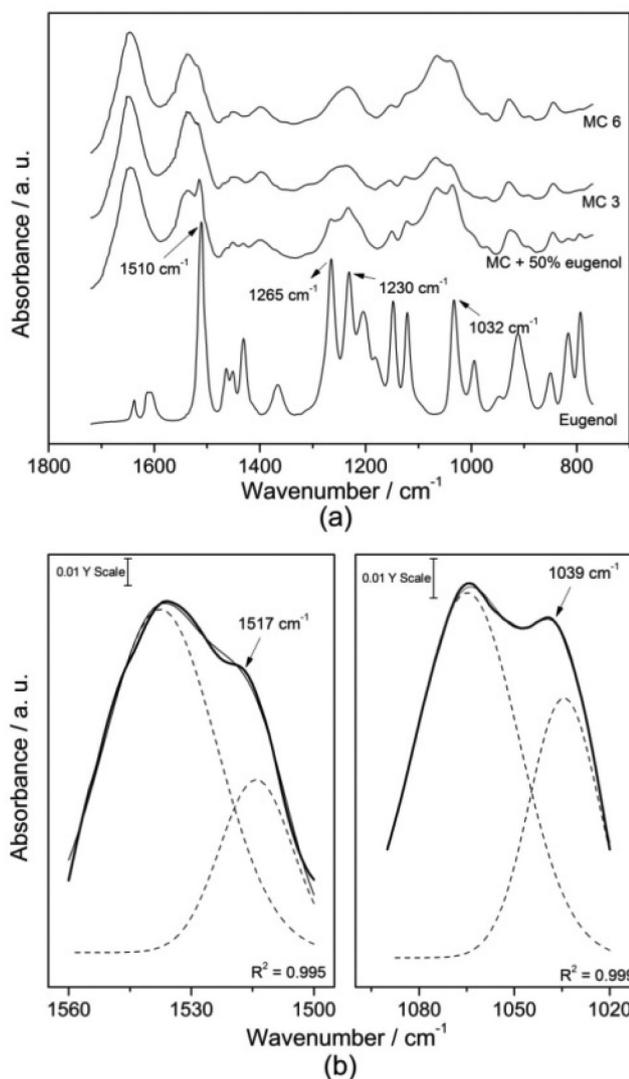


Figure 3. FTIR-ATR spectrum of (a) eugenol-loaded microcapsules assay 6 (MC 6), assay 3 (MC 3), eugenol-loaded-microcapsules assay 6 added with 50% pure eugenol (w/w) (MC + 50% eugenol), and pure eugenol; FTIR-ATR deconvolution spectrum of (b) eugenol microencapsulated in carrageenan, rice bran protein and bovine serum albumin (MC 6) in a wavenumber ranging from 1560 to 1500 cm^{-1} (left) and from 1090 to 1020 cm^{-1} (right) showing eugenol bands presence at 1517 cm^{-1} and 1039 cm^{-1} . Original FTIR-ATR spectrum (thick solid line); Gaussian curve-fit (solid line); Peaks from deconvolution analysis (dashed lines)

materials and less intense than that for pure eugenol. This result suggests the wall materials protective effect on eugenol.

For microcapsules, DTG showed four main thermal loss of weight events. The first one occurred at 100 °C for both assays, which correspond to microcapsules moisture content (about 7%). Another thermal loss of weight event was noted at 250 °C that may be from eugenol evaporation. Tanaka, Lu, Yuasa & Yamaura⁴⁸ reported loss of weight events to *k*-carrageenan, at 220 °C, however, this difference in thermal degradation temperature between carrageenan systems may be due to each system morphological conformation, dependent on processing conditions. Although both microcapsules were added with the same carrageenan amounts, MC 3 assay presented a more intense peak than MC 6. This may be due to the presence of a greater amount of non-encapsulated eugenol in the microcapsules from the MC 3 assay. The DTG peaks at 315 °C and 307 °C for MC 3 and MC 6 assays, respectively, characterize the microcapsules collapse and,

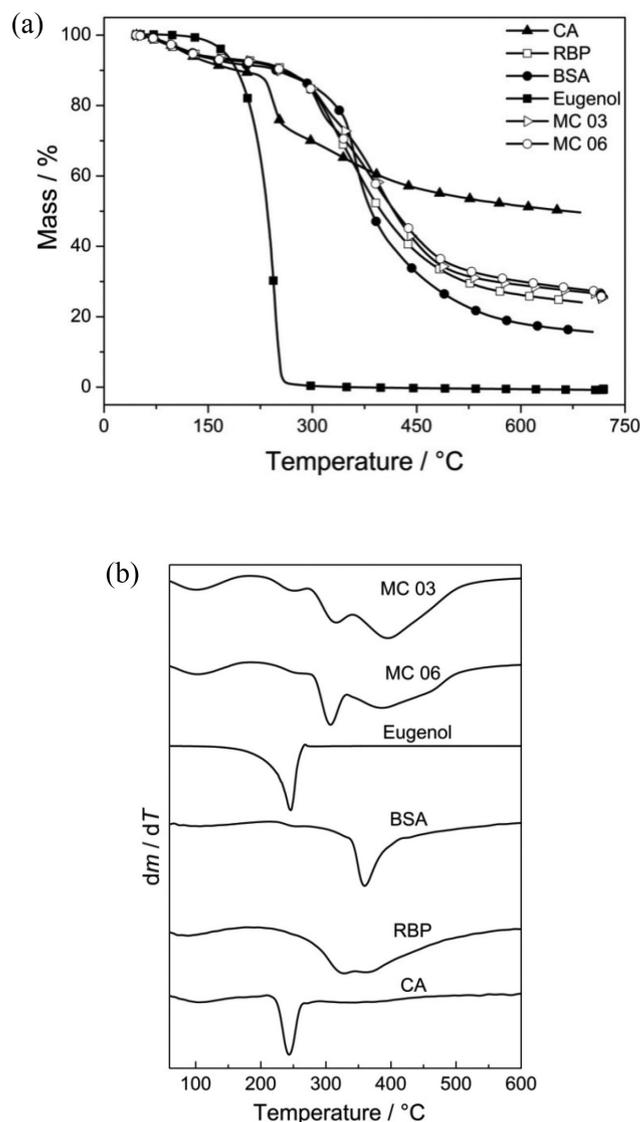


Figure 4. TG (a) and DTG (b) analyses of eugenol microencapsulated in carrageenan and bovine serum albumin (MC 3), eugenol microencapsulated in carrageenan, rice bran protein, and bovine serum albumin (MC 6), and the pure materials — carrageenan (CA), rice bran protein (RBP), bovine serum albumin (BSA), and eugenol

hence, eugenol release. Such results showed that the encapsulation process resulted in increased eugenol thermal stability inside these systems, improving eugenol retention and encapsulation efficiency.

The peak associated with microcapsules collapse was more pronounced in MC 6 assay, which represents an encapsulated eugenol rate higher than the non-encapsulated one. Microcapsules wall material thermal degradation in both assays was noted at 390 °C.

CONCLUSIONS

Simplex-centroid mixture design may be used to maximize encapsulation efficiency of eugenol-loaded microcapsules using carrageenan spray drying and protein blends as wall materials. The use of rice bran protein and bovine serum albumin with carrageenan produced microcapsules with the highest eugenol retention; this effect may be correlated to suspension stability prior to drying. Thus, the binary interaction between these proteins resulted in the highest encapsulation efficiency allowing for better conditions to obtain eugenol-loaded microcapsules, as per desirability parameters and

encapsulation efficiency response surface methodology. Optimum point microcapsules showed a spherical morphology and fissure-free, with such systems' wall surface showing a solid appearance, as well as good-film-formation particles. The FTIR-ATR and thermal analyses confirmed eugenol encapsulation, showing an eugenol predominance inside the microcapsules at temperatures above 300 °C.

ACKNOWLEDGMENTS

The authors would like to thank CAPES, CNPq, and Fundação Araucária for their financial support.

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