ISSN 0100-2945 DOI: http://dx.doi.org /10.1590/0100-29452018874

Revista Brasileira de Fruticultura

Microencapsulation of Pequi pulp oil by complex coacervation

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Abstract - Pequi pulp oil, *Caryocar brasiliense*, is rich in carotenoids, antioxidant compound easily oxidized by the presence of heat, light and oxygen. In order to improve its stability, pequi oil was microencapsulated by complex coacervation using gelatin and Arabic gum as encapsulating agents. Twenty formulations were prepared using a 2^3 central composite rotational design. The influence of temperature, stirring velocity and core material in the oil coacervation were evaluated, aiming to preserve carotenoids present in the oil. The best yield values and carotenoids content were obtained at the midpoint of the design (7.5g core, 15.000rpm and 50°C). Particles showed asymmetric distribution, with diameter ranging from 15 to 145 μm and the efficiency of the encapsulation process, obtained by the retention of oil in the microcapsule, ranged from 66.58 to 96.50%, thus demonstrating the encapsulation efficiency of this method.
Index terms: *Caryocar brasiliense*, carotenoids, retention.

Microencapsulação de óleo da polpa de Pequi por coacervação complexa

Resumo - O óleo da polpa de pequi, *Caryocar brasiliense*, é rico em carotenoides, composto antioxidante facilmente oxidado pela presença de calor, luz e oxigênio. Com o objetivo de melhorar sua estabilidade, o óleo de pequi foi microencapsulado por coacervação complexa utilizando gelatina e goma arábica como agentes encapsulantes. Vinte formulações foram preparadas a partir do delineamento composto central rotacional 2^3. Neste estudo, foram avaliados a influência da temperatura, a velocidade de agitação e o material de núcleo na coacervação, a fim de preservar os carotenoides presentes no óleo. Os melhores valores de rendimento e carotenoides foram obtidos no ponto central do delineamento (7,5 g de recheio, 15.000 rpm e 50ºC). As partículas apresentaram distribuição assimétrica com diâmetro de 15 a 145μm, e a eficiência, obtida em função do óleo retido na microcápsula, variou entre 66,58 e 96,50%, comprovando a eficácia deste método de encapsulação.
Termos para indexação: *Caryocar brasiliense*, carotenoides, retenção.

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Received: September 28, 2016.
Accepted: October 04, 2017.

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Introduction

The Cerrado Biome corresponds to 24% of the Brazilian territory, concentrating one third of the national biodiversity and 5% of the world's flora and fauna. Pequi (Caryocar brasiliense) stands out among the native fruits of the Cerrado due to its great economic value and important nutritional source, being widely used in the cuisine of the Midwestern region, especially in the state of Goiás. Pequi pulp is rich in carotenoids, phenolic compounds and unsaturated fatty acids, with predominance of oleic and palmitic acids (LIMA et al., 2007; BARRA et al., 2013).

Carotenoids are natural antioxidants that can prevent or reduce oxidative damage, since they have the capacity to react with free radicals, presenting beneficial properties to health, acting mainly in the prevention of carcinomas, cardiovascular, ophthalmological, pulmonary diseases and neurodegenerative disorders (RAO et al., 2007; LIMA et al., 2012; ALÓS et al., 2016). However, carotenoids are sensitive to adverse environmental conditions such as heat, light and oxygen (SANTANA et al., 2013), which accelerate their degradation, requiring the use of adequate techniques to preserve the bioactive action of these constituents. The effects of the deterioration of these compounds can be minimized by the microencapsulation process.

Microencapsulation provides protection to the encapsulated material, masking flavors and odors, offering the possibility of controlled release of contents with increased stability (AHN et al, 2008; AUGUSTIN et al, 2009). In this method, it is essential to choose the correct wall material. The main biopolymers used are polysaccharides (starch, maltodextrin, gum arabic, alginate, chitosan, carrageenan) and proteins (gelatin, casein, soy, wheat) (GIBBS et al., 1999; MARTINS et al., 2014).

Among the techniques used for microencapsulation, complex coacervation has been the most applied method in lipophilic substances. The process consists of the spontaneous separation of phases that occurs when oppositely charged polyelectrolytes (protein and polysaccharide) are mixed in a single aqueous medium and are influenced by pH, ionic and electrostatic force (DUCEL et al., 2004; COMUNIAN et al., 2016).

Recent scientific studies have shown the importance and the effect of microencapsulation by complex coacervation on the controlled availability of propolis extract (NORI et al., 2011) and oral drug celecoxib (CHENG et al., 2010). The stability of lutein and ascorbic acid was studied by Qv et al. (2011) and Comunian et al. (2013). Microencapsulation can be used in the preservation of pigments such as turmeric (ZUANON et al., 2013; WANG et al., 2012), in the retention of flavor and aroma of orange essential oil (JUN-CHIA et al., 2011) and xylitol (SANTOS et al., 2015). The conservation of carotenoids from cerrado fruits was studied by LESCANO et al. (2014) and Lima (2014).

In this context, the aim of this work was to evaluate carotenoid retention of microcapsules produced from pequi oil by the complex coacervation technique.

Material and Methods

Material

Pequi (*Caryocar brasiliense)* was purchased in Campo Grande (MS), latitude 20º2634 and longitude 54º3847, 532 m above sea level, during the period of December / January 2015 and 2016. Fruits were selected, sanitized, peeled and manually pulped with the aid of stainless steel knives. Pulp was dehydrated at 50ºC in dehydrator with air velocity of 0.5m / s for 24 hours. Oil was extracted from the dehydrated pulp by pressing at room temperature in an expeller type press. The oil obtained was centrifuged at 1500 rpm for 15 minutes to remove impurities.

Reagents

For the microencapsulation process, gelatin (Fluka), Arabic gum (Vetec) and hydrochloric acid (Vetec) were used. Analytical grade P.A. reagents were used for analyses.

Obtaining microcapsules

Pequi pulp oil microcapsules were prepared by the complex coacervation method in aqueous medium according to procedure described by Alvim et al. (2010) with modifications, obtaining a fine and stable emulsion of the core (oil) in the wall material solution. For this, an oil aliquot (3.3g to 11.7g) was mixed with 100mL of gelatin solution (2.5%), which was previously hydrated and heated (33.2°C to 66.8°C). The mixture was homogenized on ultra-high speed stirrer at controlled speed (6,600rpm at 23,400rpm) for one minute. Then, 100mL of Arabic gum solution (2.5%) and 400mL of deionized water were added, both heated to the same temperature as the previous mixture. After this step, the suspension pH was adjusted with HCl solution (0.1M) until it reached pH 4. The material was then cooled in an ice bath, under constant stirring until it reached 10°C, then it remained at rest, and the encapsulated particles as a fine emulsion were obtained by decantation, which was maintained at 8°C for 16 hours.

Optimization of the microencapsulation process

Experiments were carried out according to a 2^3 central composite rotational design (CCRD) to investigate the effects of three independent variables (temperature, homogenization velocity and filling) on the efficiency and yield of microencapsulation and carotenoid retention in microparticles. Table 1 shows the levels used in

the experimental design. The design consisted of 20 experiments including 6 replicates of the central point as shown in Table 1.

Experimental data were fitted to a second-order polynomial equation. Equation 1.

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3$$

Where **y** is the response (dependent variable); $\mathbf{b_0}$ is the regression constant; $\mathbf{b_1}$, $\mathbf{b_2}$ **and** $\mathbf{b_3}$ the regression coefficient for the linear term; $\mathbf{b_{11}}$, $\mathbf{b_{22}}$ and $\mathbf{b_{33}}$ the quadratic regression coefficient; $\mathbf{b_{12}}$ and $\mathbf{b_{13}}$, $\mathbf{b_{23}}$ the interaction coefficient of the terms and $\mathbf{x_1}$, $\mathbf{x_2}$ and $\mathbf{x_3}$ represent the values of independent variables (temperature, velocity and filling, respectively).

Table 1. Levels of independent variables used in the central composite rotational experimental design for the microencapsulation of pequi pulp oil.

Independent variables	Coded variables	Level				
		-1.68	-1	0	1	1.68
Temperature (°C)	X_1	33.2	40	50	60	66.8
Velocity (rpm)	X_2	6.600	10.000	15.000	20.000	23.400
Filling (g)	X_3	3.3	5	7.5	10	11.7

Yield

The yield of microcapsules was calculated from the separation of phases obtained in the microencapsulation (supernatant and sediment). After resting for 16 hours, microcapsules were centrifuged at 1,500 rpm and the supernatant collected and screened in 0.075mm mesh for complete separation of the supernatant and sediment (wet microcapsules). Sedimented microcapsules were weighed and the moisture content was determined (AOAC, 2000) to calculate the yield by Equation 2.

$$R\ (\%) = \frac{MS_{baseseca}}{MTI_{baseseca}} \times 100$$

Where **R** is the yield of microcapsules (%), $\mathbf{MS_{dry\ basis}}$ is the amount of sedimented microcapsule (g) and $\mathbf{MTI_{dry\ basis}}$ the initial theoretical mass consisting of Arabic gum + gelatin + oil (g).

Efficiency

The encapsulation efficiency was calculated from the amount of oil present in one gram of microcapsule (dry basis) in relation to the amount of oil initially inserted in the microencapsulation process by Equation 3 (BUENO et al., 2011).

$$E\ (\%) = \frac{OEM}{OIM} \times 100$$

Where **E** is the encapsulation efficiency (%), **OEM** is the amount of oil extracted from microcapsules (g) and **OIM** is the initial amount of oil used in the production of microcapsules (g).

Total carotenoids

Total carotenoids were quantified in the pequi pulp oil and in wet microcapsules according to methodology described by Rodriguez-Amaya (2001) based on extraction with acetone and separation in petroleum ether. Samples (2.0g) were macerated with 0.5g of celite, and sufficient amount of acetone at 10ºC until carotenoids were completely extracted in mortar. The extract obtained was vacuum filtered and transferred to a separatory funnel containing 40mL of petroleum ether. Distilled water was incorporated into the mixture giving two phases: (carotenoids + petroleum ether and water + acetone). Acetone was removed from the mixture with water. The extract (carotenoids + petroleum ether) was transferred to a 50mL volumetric flask and the volume filled with petroleum ether. The solution absorbance was determined in a UV-VIS spectrophotometer (Biochrom, model Libra S60PC) at 450 nm and petroleum ether used as white. Equation 4 informs the percentage of carotenoids in 1g of sample.

$$C\left(\frac{\mu g}{g}\right) = \frac{\lambda_{max} \times d \times 10^4}{\varepsilon \times m}$$

Where **λmax** is the maximum absorbance (nm), **d** the sample dilution (ml), **ε** is the absorption coefficient of β-carotene in petroleum ether (2592) and **m** the sample mass (g).

Size and distribution of microcapsules

An optical microscope coupled to a photographic camera (Nikon Eclipse - 200) was used to verify the formation of microcapsules. An aliquot of microcapsules was diluted in distilled water and the suspension was evaluated. Two hundred microcapsules were analyzed for each sample. The size was determined from images obtained by the Image Pro Plus 4.0 software.

Statistical analysis

Experiments were carried out according to the central composite rotational design in triplicate. Differences between mean values were determined by

the analysis of variance (ANOVA) and the comparison of means by the Tukey's test ($p \leq 0.05$) using the Statistica 7.0 program (SAS INSTITUTE, 2004).

Results and discussion

Yield

The yield values of the process, microencapsulation efficiency and carotenoid retention in microcapsules are shown in Table 2. The values obtained for the yield of microcapsule formation in treatments varied from 67.07% to 97.07% according to the conditions applied (Table 2).

These values were similar to those obtained by Siow et al. (2013) in microencapsulation by complex coacervation of garlic oil using gelatin and Arabic gum as encapsulating agents. According to the authors, this result may be related to the proportion of encapsulating agents used in the process. When one of the colloids (gum or gelatin) is in excess in the system, the presence of unneutralized charges will reduce the attraction between the two colloids, thereby reducing the yield of coacervates.

Yield indicates that the interaction of encapsulating agents (Arabic gum + gelatin) was effective in the formation of microcapsules. The highest yield was obtained in center point assays, that is, in the process condition carried out at 50°C with stirring at 15,000rpm and 7.5g of filling (pequi oil), obtaining on average 96.71%. These results show the influence of temperature, homogenization velocity and filling in the formation of pequi oil microcapsules.

Table 3 shows the regression coefficients, the F value and the determination coefficient (R^2) for the yield. Considering the estimates of each parameter, the second-order model was predictive (Equation 5) with determination coefficient (R^2) of 98%. The results of the statistical analysis are also presented by the Pareto graph (Figure 1), where the quadratic terms of temperature, filling and velocity and linear term of the filling, as well as the linear velocity-filling and temperature-velocity interaction were significant ($p \leq 0.05$).

$$R(\%) = -272{,}11 + 8{,}46 \times A - 0{,}08 \times A^2 + 0{,}01 \times B - 2{,}8 \times 10^{-7} \times B^2 - 2{,}1 \times 10^{-5} \times A \times B - 7{,}9 \times 10^{-3} \times 7{,}5 \times A - 1{,}2 \times 10^{-4} \times 7{,}5 \times B + 88{,}74$$

Table 2. Responses of the central composite rotational experimental design for pequi oil microcapsule using gum arabic and gelatin as encapsulants.

Test	A (ºC)	B (rpm)	C (g)	Yield (%)	Efficiency (%)	Carotenoids (µg/gms) *
1	40	10.000	5	67.07	66.58	60.30
2	40	10.000	10	79.12	78.06	40.95
3	40	20.000	5	73.91	74.68	46.86
4	40	20.000	10	76.70	75.64	29.27
5	60	10.000	5	72.45	71.80	31.84
6	60	10.000	10	80.29	79.96	49.32
7	60	20.000	5	71.65	71.99	25.83
8	60	20.000	10	77.06	76.26	24.96
9	33,2	15.000	7.5	74.75	75.20	25.25
10	66,8	15.000	7.5	72.30	72.42	24.73
11	50	6.600	7.5	77.70	76.83	23.95
12	50	23.400	7.5	75.67	76.25	21.74
13	50	15.000	3.3	73.01	74.28	44.15
14	50	15.000	11.7	79.49	79.06	23.85
15 (0)	50	15.000	7.5	96.76	96.24	192.15
16 (0)	50	15.000	7.5	97.07	96.86	191.60
17 (0)	50	15.000	7.5	96.40	96.70	191.48
18 (0)	50	15.000	7.5	96.91	96.34	191.96
19 (0)	50	15.000	7.5	96.45	96.68	192.10
20 (0)	50	15.000	7.5	96.67	96.50	192.04

*A, temperature (° C); B, homogenization velocity (rpm); C, filling (g); 0, central point *, expressed in dry mass.

However, the formation of microcapsules does not necessarily mean that they present the core filled of the substance to be encapsulated, so the encapsulation efficiency must be considered.

Efficiency

The encapsulation efficiency corroborated the yield, presenting values between 66.58 and 96.86% (Table 2). According to Assis et al. (2012) and Bakry et al. (2016), coacervation is a technique that has high encapsulation efficiency (up to 99%). Prata et al. (2015) obtained 91.8% efficiency in the microencapsulation of vetiver essential oil using gelatin and Arabic gum as encapsulants and Santos et al. (2015) in the microencapsulation of xylitol, obtaining efficiency from 31.42 to 62.94% with the same agents used in this study.

Variation in efficiency can be attributed to the active-material ratio, according to Zuanon et al. (2013), smaller amounts of filling increase the possibility of polymers covering the drop of the material, allowing greater efficiency. In this study, the influence of the three independent variables presented values of approximately 97% in central point assays (50°C, 15,000rpm and 7.5g of filling).

The microencapsulation efficiency was influenced by the quadratic model of temperature, velocity and filling (Table 4), as well as the interaction of the temperature-velocity and velocity-filling linear term. The efficiency model (E) presented good fit to experimental data. Considering the significant coefficients, the predictive model was obtained with adjustment of 98% (Equation 6).

$$E = -205{,}35 + 6{,}79 \times A - 0{,}08 \times A^2 + 0{,}01 \times B - 2{,}8 \times 10^{-7} \times B^2 - 2{,}3 \times 10^{-5} \times \times A - 1{,}4 \times 10^{-4} \times 7{,}5 \times B + 86{,}83$$

Figure 2 presents the experimental results and those predicted by the adjusted model, showing good agreement among them as expected by the results of the analysis of variance (Table 4).

Carotenoids

Carotenoid retention in wet microcapsules ranged from 21.74 to 192.15µg / gms according to Table 2. The carotenoid content in the crude pequi oil was 333.03µg / gms. Considering the oil encapsulation efficiency, it could be verified that there was degradation of carotenoids from 3.86% to 89.77%. The lowest retention values were verified for assays that presented higher homogenization velocity, probably due to the direct exposure to oxygen during the emulsion formation caused by the high velocity, which causes the oxidation of this biocomposite.

The regression analysis of results (Table 5) shows that all variables studied (temperature, homogenization velocity and filling) were significant ($p \leq 0.05$), generating the predictive model for carotenoids (Car) presented in (Equation 7). The model adjusted to experimental data with determination coefficient (R^2) of 98%.

$$Car = -2025{,}74 + 52{,}57 \times A - 0{,}54 \times A^2 + 0{,}068 \times B - 2{,}2 \times 10^{-6} - 1{,}3 \times 10^{-5} \times A \times B + 0{,}27 \times 7{,}5 \times A - 1{,}6 \times 10^{-4} \times B + 370{,}1$$

Table 3. Regression parameters for the analysis of variance ANOVA of the microencapsulation yield of pequi oil (*Caryocar brasiliense*).

Factor	SQ	DF	QM	F	p
(1) Temperature (L)	0.042	1	0.042	0.048	0.828
Temperature (Q)	1876.699	1	1876.699	2132.520	≤0.05
(2) Velocity (L)	1.337	1	1.337	1.519	0.229
Velocity (Q)	1392.399	1	1392.399	1582.204	≤0.05
(3) Filling (L)	222.672	1	222.672	253.026	≤0.05
Filling ((Q)	1455.460	1	1455.460	1653.860	≤0.05
1L 2L	17.808	1	17.808	20.236	≤0.05
1L 3L	0.624	1	0.624	0.709	0.407
2L 3L	34.164	1	34.164	38.821	≤0.05
Lack of adjustment	44.974	5	8.995	10.221	≤0.05
Pure error	22.001	25	0.880		
Total SS	4297.590	39			
R^2	0.9844				

*SQ, sum of squares; DF, degrees of freedom; QM, quadratic mean; F Fisher parameter for the significance test of effects; p (significance level ≤0.05).

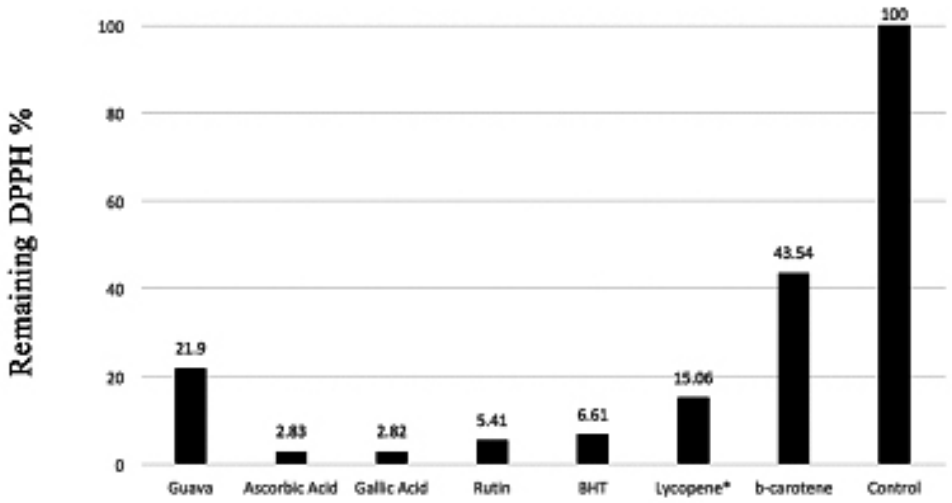

Figure 1. Pareto plot, effects and interactions of temperature (° C), filling (g) and homogenization velocity (rpm) on the yield of pequi oil microcapsules.

Table 4. Regression parameters for the ANOVA analysis of variance of the efficiency of pequi oil microencapsulation (*Caryocar brasiliense*)

Factor	SQ	DF	QM	F	p
(1) Temperature (L)	0.020	1	0.020	0.031	0.862
Temperature (Q)	1864.557	1	1864.557	2917.342	≤0.05
(2) Velocity (L)	0.208	1	0.208	0.325	0.573
Velocity (Q)	1444.047	1	1444.047	2259.400	≤0.05
(3) Filling (L)	158.626	1	158.626	248.192	≤0.05
Filling (Q)	1424.986	1	1424.986	2229.576	≤0.05
1L 2L	21.045	1	21.045	32.928	≤0.05
1L 3L	0.000	1	0.000	0.000	0.997
2L 3L	51.876	1	51.876	81.167	≤0.05
Lack of adjustment	45.272	5	9.054	14.167	≤0.05
Pure error	15.978	25	0.639		
Total SS	4253.893	39			
R^2	0.9856				

*SQ, sum of squares; DF, degrees of freedom; QM, quadratic mean; F Fisher parameter for the significance test of effects; p (significance level ≤0.05).

The response surface and the contour chart of carotenoid retention in microparticles are shown in Figure 3 (a, b). By the surface analysis of response generated by the model, it was verified that higher carotenoid retentions were observed at temperature between 35ºC and 65ºC together with homogenization velocity between 10.000rpm and 20.000rpm. Conditions similar to this study were presented by Lescano et al. (2014) in the microencapsulation of "bocaiuva" oil using the same encapsulating agents. According to the author, high temperatures and the incorporation of air during the stirring process contribute to the degradation of carotenoids. In another study, Qv et al. (2011) observed that increasing the temperature in the encapsulation process reduced the rate of lutein retention in microcapsules formed with gelatin and Arabic gum, corroborating the present study.

The region of highest carotenoid retention (Figure 3a, b) is located at the central point of the design, where the process conditions were 50ºC, 15,000rpm and 7.5g of filling, observed on the response surface and on the contour graph of by the red color intensity.

Particle Size

The size of wet microcapsules containing pequi oil is shown in Figure 4. An asymmetric particle size distribution is observed. The diameter of microcapsules ranged from 15 to 145 μm, and 71.06% of microcapsules had size between 15 and 34 μm. According to Favaro-Trindade et

al. (2008) the expected values for microcapsules obtained by complex coacervation range from 1 to 500 µm.

Diameters of (50-100µm) and (45.3-296.5µm) were observed in the microencapsulation of broccoli (SANCHEZ et al., 2016) and in the soybean oil - stearidonic acid complex (IFEDUBA et al., 2015), respectively. The particle size can be influenced by several factors such as stirring rate, viscosity, filling-polymer content, pH, cooling rate and drying (LAMPRECHT et al., 2001; NAKAGAWA et al., 2004; KAUSHIK et al. 2015). Smaller particles may be attributed to encapsulating agent particles that were not encapsulated (Tonon et al., 2011). However, Lee et al. (1999) points out that smaller sized microcapsules have larger surface area and thinner membrane, making the core material easier to diffuse through the microcapsule membrane.

According to Kaushik et al. (2015) the recommended particle size for application in food products should be less than 100 µm in order to avoid sensory perception in the mouth. For application in drugs, particle size should be less than 200µm (AZAGHESWARI et al., 2015). Therefore, particles obtained in this study can be used in both food and drugs.

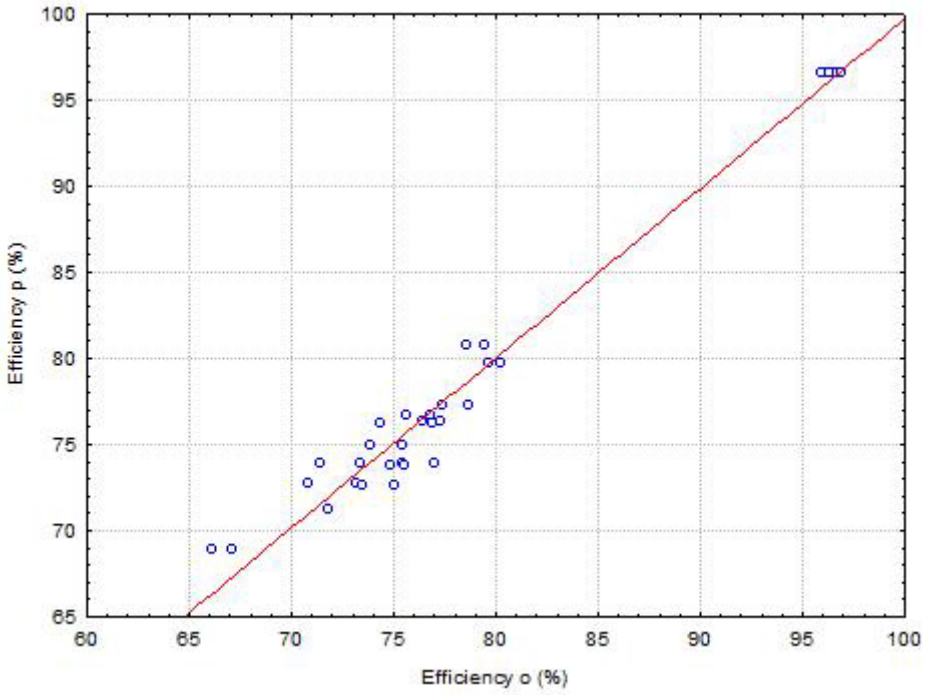

Figure 2. Graph of predicted and experimental values of the encapsulation efficiency of pequi oil. Efficiency p (%): efficiency values predicted by the mathematical model; Efficiency o (%): efficiency values experimentally obtained.

Table 5. Regression parameters for the analysis of variance ANOVA of carotenoids from pequi oil microencapsulation (*Caryocar brasiliense*).

Factor	SQ	DF	QM	F	p
(1) Temperature (L)	314.2	1	314.19	806.3	≤0,05
Temperature (Q)	85430.0	1	85430.03	219238.5	≤0,05
(2) Velocity (L)	513.7	1	513.66	1318.2	≤0,05
Velocity (Q)	87827.9	1	87827.95	225392.3	≤0,05
(3) Filling (L)	434.2	1	434.23	1114.4	≤0,05
Filling (Q)	75720.1	1	75720.15	194320.1	≤0,05
1L 2L	6.9	1	6.88	17.6	≤0,05
1L 3L	716.5	1	716.50	1838.7	≤0,05
2L 3L	68.8	1	68.77	176.5	≤0,05
Lack of adjustment	3658.3	5	731.67	1877.7	≤0,05
Pure error	9.7	25	0.39		
Total SS	213855.4	39			
R^2	0.9828				

*SQ, sum of squares; DF, degrees of freedom; QM, quadratic mean; F Fisher parameter for the significance test of effects; p (significance level ≤0.05)

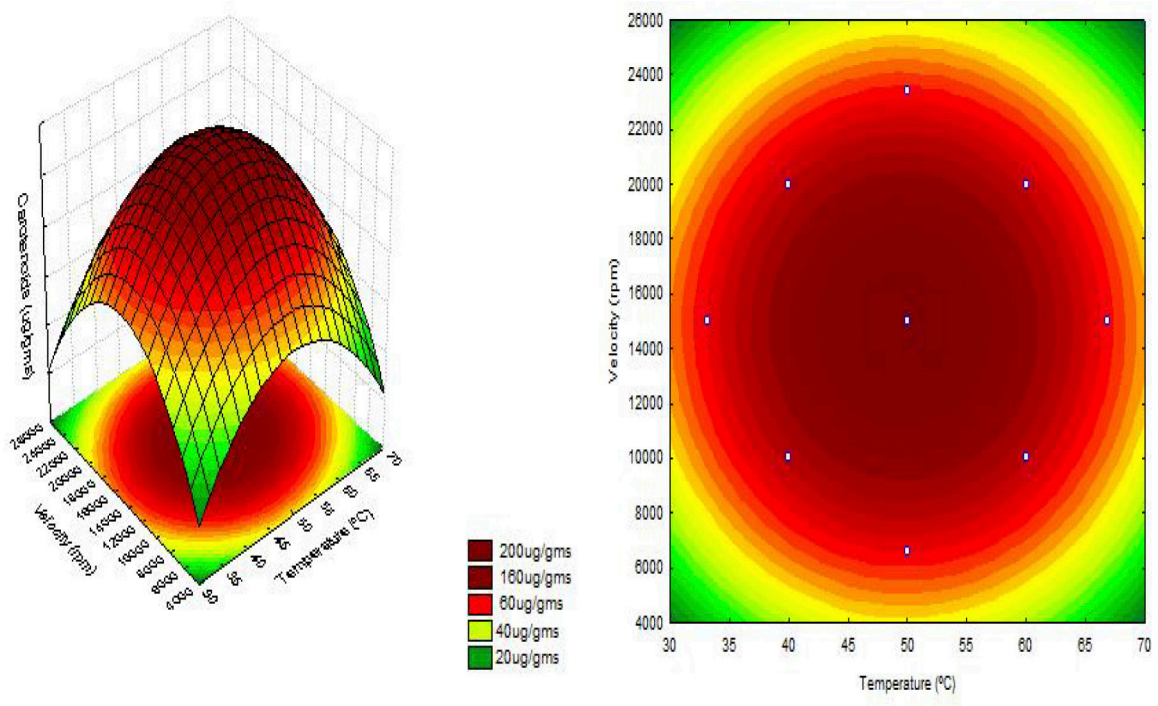

Figure 3. Response surface (a) and contour graph (b) of velocity, temperature and filling interaction on the influence of carotenoid degradation on pequi pulp oil microcapsules.

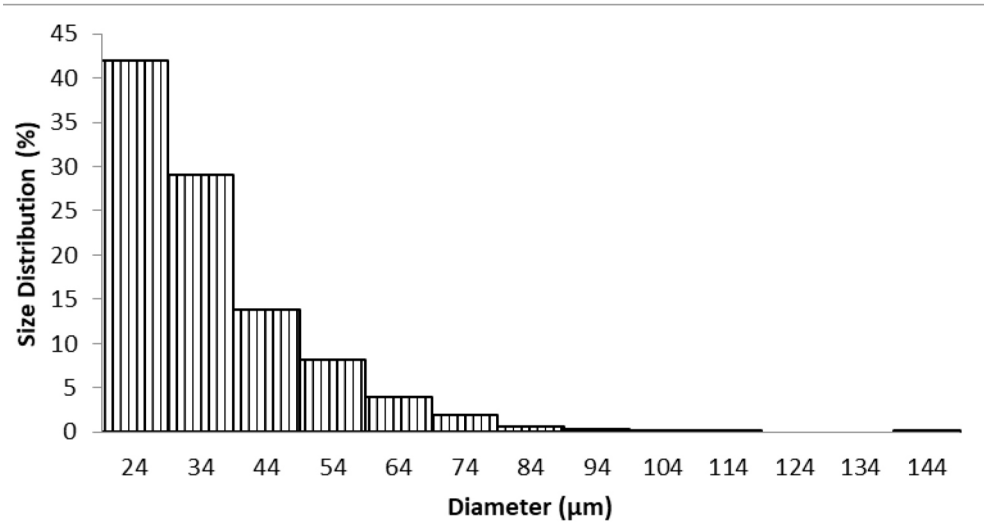

Figure 4. Particle size distribution.

Conclusions

The results presented in this study show that it is possible to microencapsulate pequi oil by complex coacervation. The independent variables of the microencapsulation process had a significant influence on yield, efficiency and carotenoid retention. The models generated by the central composite rotational design were predictive with correlation coefficients of 98%. The size of particles presented asymmetric distribution within the range established in literature. From these results, future studies should be carried out to evaluate the stability of pequi oil microcapsules for their application in the food and health sectors.

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ERRATUM

http://dx.doi.org/10.1590/0100-29452018874erratum

Erratum of article: Justi, P. N., Sanjinez-Argandoña, E. J., & Macedo, M. L. R. Microencapsulation of Pequi pulp oil by complex coacervation. *Rev. Bras. Frutic., 40*(2), e-874. http://dx.doi.org/10.1590/0100-29452018874

In the **page 6**, figure 1

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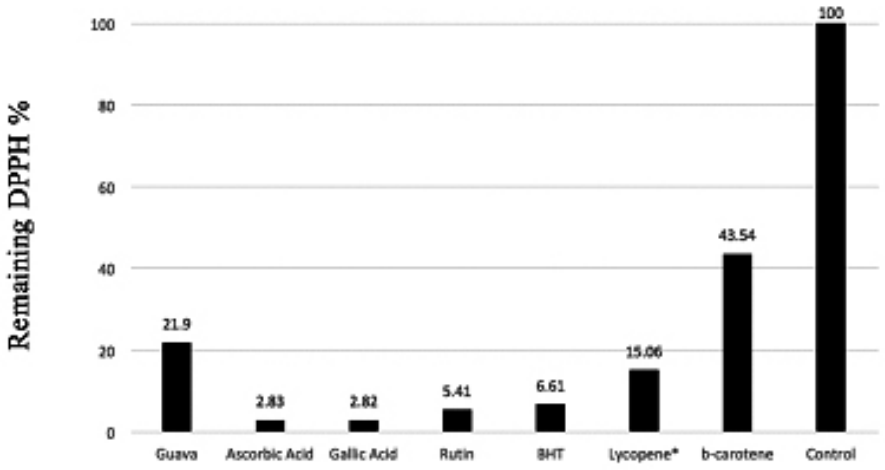

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