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# Encapsulation of bixin in starch matrix: Optimization from Ultrasound-assisted microencapsulation and alkaline method.

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## Abstract

Encapsulation of bixin was carried out by ultrasound treatment and alkaline method, using amylose extracted from cassava starch and high-amylose corn starch. The effect of preparation condition on bixin encapsulate was determine by employing UV-vis spectroscopy and modeled using the response surface methodology. Bixin content obtained using the alkaline method ranged from 910 to 6475.25 µg /g for encapsulate bixin inside of matrix and from 96.0 to 10330.1 µg/g for bixin on surface. Bixin encapsulate using ultrasound treatment ranged between 1234.0 to 3787.68 µg /g for bixin inside of matrix and between 267.26 to 6210.43 µg /g for bixin on the surface. Encapsulation efficiency ranged between 13.10% to 62.12% and 17.27% to 94.48% using ultrasound and the alkaline method respectively. The optimum conditions were found as 2% Amylose, 150 W and 20 min for ultrasound treatment, and 2% amylose from cassava with protein at 68°C for the alkaline method.

**Keywords:** Whey protein, RSM, optimization, carotenoids, cassava.

## Introduction

Bixin represents about 80% of carotenoids content in the annatto (*Bixa Orellana*) seeds. Its structure contains a system of double bonds, which affect their stability and makes them susceptible to physical and oxidative degradation induced during the processing and storage (Montenegro, Rios, Mercadante, Nazareno, & Borsarelli, 2004; Rios, Borsarelli, & Adriana, 2005). Microencapsulation is a technology by which of bioactive molecules are packed inside of carrier or wall material, improving their stability or solubility (Fathi, Martín, & McClements, 2014). Generally, for development of microencapsulation system are used drying methodologies, which becomes the bioactive compound in powder. These types of technologies commonly use carbohydrates or proteins as wall materials. Indeed, in the delivery systems preparation can be used starch, cellulose, pectin, guar gum, chitosan, alginate, dextrin, cyclodextrin, gums and their combination (Fathi et al., 2014; Marcolino, Zanin, Durrant, Benassi, & Matioli, 2011). In the case of carotenoid as bixin, the microencapsulation improve their stability against light and enhance their solubility (Lyng, Passos, & Fontana, 2005). In the last few years, preference of consumers by oral consume of active compound has notably increased, which motive the continuous search of technologies and delivery systems that improve the bio-disponibility of bioactive agents (Braithwaite et al., 2014; Giridhar,

Venugopalan, & Parimalan, 2014; McClements & Li, 2010). In our previous studies about bixin microencapsulation, we explored the possibility of preparation of V-amylose complex by ultrasound treatment and using the alkaline method. The results indicated that there is no formation of V-amylose complexes. Nevertheless, the results obtained of both methods suggested that is possible to obtain a good encapsulation matrix, resulting in a system with several delivery patterns. For this reason, this study aimed to choose the best condition and to compare these two methodologies encapsulation. The best condition was selected using the response surface methodology (RSM). The RSM is a statistical tool based on in the fitting of experimental data to a polynomial equation. The model provides by RSM describe and predict the statistical behavior of experimental data (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Gilmour, 2006). The RSM is one of the most common optimization methods applicable to design, improvement, and formulation of new products and also development of existing products properties (Bas & Boyacı, 2007). It is useful in the resolve of optimization problems in the engineering of food. The RSM has advantage over methodologies such as the optimization of one-variable-at-a-time, due it is possible to determine the effects and interactions of the parameters in the study (Bezerra et al., 2008). For this reason, The RSM was used to determine the best conditions in the microencapsulation of bixin.

## Material and Methods

High-amylose (72 % of amylose, according to manufacturer) corn starch (Hylon VII) was obtained from Ingredion Brasil Ing. Ind. Ltda (Mogi Guaçu, SP, Brazil). Bixin was obtained from BKG (Adicon, Brazil). Whey protein concentrate (WP) was obtained from Alibra ingredients Ltda (SP, Brazil). Sodium hydroxide (NaOH) was supplied by Synth (Diadema, Brazil), hydrochloric acid was provided by Quimis (Diadema, Brazil), and pancreatin was provided by Sigma-Aldrich (St. Louis, MO). Starch suspensions were prepared using deionized water. All chemicals were of analytical grade.

## Fractionation of cassava starch

Amylose was isolated from cassava starch by precipitation with ethanol according to methodology proposed by Mua and Jackson (1995). A dispersion of cassava starch (2% w/w) was heated at 70 °C for 20 min. The solution obtained was cooled to 40 °C and centrifuged to 3500×g for 10 min. The supernatant was separated and the amylose was precipitated using ethanol (95% v/v). This precipitate was separated by centrifugation to 1500×g for 3 min and stored at 8 °C until used.

## Encapsulation using the alkaline method

One hundred and fifty grams of the starch suspension Hylon VII or amylose fractionation of cassava starch was mixed with bixin (0.1 g) in 0.01 M KOH solution (50 g) at temperatures and concentration according to the experimental design shown in Table 2, followed by precipitation at pH 4.5 using 0.01M HCl. The precipitate was separated by centrifugation at 3500×g for 10 min, was cooled at -18 °C for 12 hours and lyophilized. When the use of protein (WP) was necessary, it was added to the starch suspension according to table 2.

**Encapsulation by ultrasound treatment**

Ninety grams of high-amylose corn starch suspension (GS) was mixed with bixin (0.2 g) in 0.01 M KOH solution (50 g) at 60°C for 1 min. This mixture was subjected to ultrasound treatment according to the experimental design shown in Table 3 at 78 °C in an ultrasonic homogenizer (Omni Ruptor 4000, Omni International, Marietta, USA) equipped with a standard probe 1.9 mm in diameter. The pH was adjusted to 4.7 using 0.01 M HCL solution and the samples were stored at 47 °C for 12 h. After that, the gels formed were frozen at -18 °C and lyophilized.

**Surface and bixin encapsulation**

The surface and encapsulated bixin content were determined according to Lalush et al., (2005) and Sutter et al., (2007) with modifications. Surface bixin was determined by washing 0.010 g of the complex with 4 mL of acetone in a test tube and shaking in a vortex for 2 min. After sedimentation, the powder was separated and the concentration of bixin in the acetone was measured spectrophotometrically at 457 nm. Encapsulated bixin in the remaining powder was determined by degradation of the complex with pancreatin. The powder was incubated in 4 mL of pancreatin solution at 37 °C for 36 h. Then, bixin was extracted with 7 mL of acetone, centrifuged, filtered and quantified spectrophotometrically at 457 nm. This wavelength was found to correspond to the maximum absorbance of the used bixin, in the spectrum range of 200 to 800 nm. For preparing the pancreatin solution, 0.18 g of pancreatin was dissolved in 20 mL of phosphate buffer 20 mM (pH 7.0) containing NaCl (0.04 % w/w). This solution was centrifuged (9000×g, 10 min), the supernatant was filtered and used for the test. Bixin content in the complex (BC) was calculated for each formulation as µg of bixin per g (Rahmalia, Fabre, Usman, & Mouloungui, 2014; Rodriguez-Amaya, 2001). The analyses were carried out in duplicate.
The microencapsulation efficiency (E*e*) was obtained from the equation 1.

$$Ee\ (\%) = \frac{C_i}{C_T} x\ 100 \qquad (1)$$

Where Ci encapsulate bixin content inside starch matrix and $C_T$ total bixin content in the starch matrix.

**Color analysis**

The color of the freeze-dried samples was determined using a ColorFlex model 45/0 spectrophotometer (Hunterlab, USA) with the D65 illuminant and observer at $10^o$. The 4.10 version of Universal software was used to determine the absolute values of $L^*$, $a^*$, and $b^*$. The system used for specification of color was CIELAB. Values of $L^*$ (lightness) range between zero (black) and one hundred (white), $a^*$ between $-a^*$ (green) and $+a^*$ (red), and $b^*$ between $-b^*$ (blue) and $+b^*$ (yellow). The chroma (C*), which expresses the degree of intensity or saturation of the color (equation 2), and the hue angle (°*hue*), which represents the tonality of the color (equation 3), were calculated. The analyses were carried out in triplicate.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

$$°hue = arctg\left(\frac{b^*}{a^*}\right) \quad (3)$$

**Experimental design**

Response surface methodology was used for modelling response (encapsulate bixin inside of matrix, encapsulate bixin outside of matrix and color). The determination of the effects of each factor on the microencapsulation of bixin was performed using d-optimal design. In the microencapsulation by alkaline method were used two numerical factors: the concentration of amylose between 2 to 8%, heating temperature between 60 to 90 °C, and two categorical factors: Hylon VII or cassava starch, presence of whey protein. In the microencapsulation by ultrasound were used three numerical factors: concentration of amylose between 2% to 8%, ultrasound power between 50% (150W) to 100% (300 W) of the maximum, time of ultrasound treatment between 20 to 40 min. The experimental design consisted of 13 and 10 points for the model in the alkaline method and by ultrasound respectively, 5 points to determine lack of fit, and 5 replicates. Statistical analysis of the experimental design was defined for a significance level of α=5%, The optimum levels of independent values were analyzed by using desirability function method. Design-Expert Software 8.0.5 (Statease Inc., Minneapolis, USA) was used for regression, analysis of variance (ANOVA) and optimization. The behavior of factors for predicting the response variables is explained by the equation 4.

$$Y = \beta_0 + \beta_A A + \beta_B B + \beta_C C + \beta_D D + \beta_{A^2} A^2 + \beta_{B^2} B^2 + \beta_{C^2} C^2 + \beta_{D^2} D^2$$
$$+\beta_{AB} AB + \beta_{AC} AC + \beta_{AD} AD + \beta_{BC} BC + \beta_{BD} BD + \beta_{CD} CD \quad (4)$$

where Y is the response, $\beta_0$ is the intercept, $\beta_A$, $\beta_B, \beta_C, \beta_D$ are the coefficients of the linear terms, $\beta_{A^2}, \beta_{B^2}, \beta_{C^2}, \beta_{D^2}$ are the coefficients of the quadratic terms, and $\beta_{AB}$, $\beta_{AC}, \beta_{AD}, \beta_{BC}, \beta_{BD}, \beta_{CD}$ are the coefficients of interactive terms.

**Results and discussion**

**Surface and bixin encapsulation**

Table 1 shows the values of bixin content on the surface, inside the starch matrix and encapsulation efficiency obtained using the alkaline method. Table 2 shows the coefficient of the model according to equation 4. An analysis of variance (ANOVA) indicated that the factors amylose concentration, heating temperature, type of starch used, presence of protein whey and interaction terms were all significant ($p < 0.05$). Presence of protein do not have significant on $L^*$ parameters. The determination coefficient $R^2_{adj}$ is reasonably close to $R^2_{pred}$, with a difference lower than 0.2. The determination coefficient $R^2_{adj}$ of models was higher than 0.83 in both methods. This indicates that the models explain more than 83% of data of experimental design. The model has non-significant lack of fit, for this reason, can be used for predictive proposes.

**Table 1.** Surface and bixin encapsulation and encapsulation efficiency obtained using the alkaline method.

| Run | Factors | | | | Bixin content outside of matrix (µg/g) | Bixin content inside of matrix (µg/g) | Total con (µ |
|---|---|---|---|---|---|---|---|
| | **A**-Amylose (%) | **B**-Temperature (°C) | **C**-Type of starch | **D**-Whey protein | | | |
| 1 | 2.5 | 63 | Hylon VII | Presence | 1736.29 | 2693.53 | 442 |
| 2 | 5.5 | 78 | Hylon VII | Absence | 2859.7 | 967.16 | 382 |
| 3 | 2.1 | 78 | Cassava | Absence | 10330.1 | 5685.44 | 160 |
| 4 | 5.6 | 60 | Hylon VII | Presence | 1890.91 | 2133.92 | 402 |
| 5 | 4.4 | 73 | Cassava | Presence | 1156.22 | 5140.9 | 629 |
| 6 | 8 | 60 | Hylon VII | Absence | 2488.14 | 1037.29 | 352 |
| 7 | 5 | 84 | Hylon VII | Presence | 1226.48 | 2374.03 | 360 |
| 8 | 6.9 | 75 | Cassava | Absence | 2180 | 3252.91 | 543 |
| 9 | 2.1 | 78 | Cassava | Absence | 9177.66 | 5445.69 | 146 |
| 10 | 2 | 90 | Hylon VII | Absence | 4266.67 | 1135.14 | 540 |
| 11 | 5.6 | 60 | Cassava | Absence | 96 | 1644 | 17 |
| 12 | 7.9 | 72 | Cassava | Presence | 612.77 | 2471.48 | 308 |
| 13 | 7.9 | 72 | Cassava | Presence | 551.72 | 2648.28 | 32 |
| 14 | 5.6 | 60 | Hylon VII | Presence | 2768.79 | 2289.36 | 505 |
| 15 | 7.4 | 90 | Hylon VII | Absence | 584.52 | 1621 | 220 |
| 16 | 8 | 90 | Hylon VII | Presence | 712.38 | 2106.55 | 281 |
| 17 | 2 | 60 | Cassava | Presence | 3190.68 | 5338.25 | 852 |
| 18 | 2.1 | 78 | Hylon VII | Presence | 3402.62 | 2280.55 | 568 |
| 19 | 5.5 | 78 | Hylon VII | Absence | 3076.62 | 910 | 398 |
| 20 | 4.4 | 90 | Cassava | Presence | 5098.04 | 6100.94 | 111 |
| 21 | 2 | 60 | Hylon VII | Absence | 4589.15 | 958.14 | 554 |
| 22 | 8 | 90 | Cassava | Absence | 1165.05 | 5054.56 | 621 |
| 23 | 4.4 | 90 | Cassava | Presence | 5980.2 | 6475.25 | 124 |

**Table 2.** Coefficients of the linear, quadratic and interactive terms for the model in the encapsulation suing the alkaline method.

| | Bixin content inside of matrix | Bixin content outside of matrix | *L** | *a** | *b** | °*hue* |
|---|---|---|---|---|---|---|
| $\beta_0$ | 2895.7 | 3023.2 | 44.7 | 32.3 | 38.2 | 48.5 |
| $\beta_A$ | -783.1 | -2216.3 | 4.2 | 0.1 | 4.0 | 3.7 |
| $\beta_B$ | 871.1 | 930.4 | -6.0 | 0.8 | -2.9 | -3.5 |
| $\beta_C$ | -1353.4 | -431.8 | 10.0 | -0.3 | 6.8 | 5.7 |
| $\beta_D$ | 455.7 | -578.7 | n.s | 0.4 | 3.4 | 2.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $\beta_{AB}$ | 209.2 | -648.1 | n.s | 1.2 | 1.0 | n.s |
| $\beta_{AC}$ | 753.5 | 1462.7 | n.s | -3.2 | -4.9 | -2.1 |
| $\beta_{AD}$ | -260.3 | 734.6 | n.s | -2.4 | -1.0 | 0.9 |
| $\beta_{BC}$ | -800.1 | -1546.4 | n.s | 0.8 | 4.2 | 3.3 |
| $\beta_{BD}$ | -210.1 | 25.5 | n.s | n.s | n.s | n.s |
| $\beta_{CD}$ | 126.2 | 115.0 | n.s | -1.9 | n.s | n.s |
| $\beta_{A}^2$ | 131.5 | 844.2 | n.s | n.s | -2.1 | n.s |
| $\beta_{B}^2$ | 65.3 | -787.5 | n.s | n.s | 2.1 | n.s |
| $\beta_{C}^2$ | n.s | n.s | n.s | n.s | -2.7 | n.s |
| $\beta_{D}^2$ | n.s | n.s | n.s | n.s | n.s | n.s |
| $R^{2pred}$ | 0.83 | 0.83 | 0.85 | 0.92 | 0.99 | 0.95 |
| $R^{2adj}$ | 0.98 | 0.89 | 0.82 | 0.97 | 0.98 | 0.98 |
| C.V. % | 8.35 | 28.20 | 12.0 | 1.82 | 2.63 | 2.52 |
| Press | 1.07e7' | 1.07e8' | 838.40 | 23.06 | 60.02 | 57.13 |
| Model (p-value) | < 0.0001 | < 0.0001 | <0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

n.s: non-significant term

The encapsulate bixin is represented by two fractions. The first located on the surface and another remains inside of the wall material. The last of them probably is protected against from heat and gastrointestinal system action. Response values (minimum and maximum) from the experimental design using the alkaline method ranged from 910 µg /g to 6475.25 µg /g for encapsulate bixin inside of matrix, from 96.0 µg/g to 10330.1 µg/g for bixin on the surface. It was obtained the higher encapsulate bixin content under the combination of factors: temperature 90°C and cassava amylose with protein.

In general, the encapsulation of bixin using alkaline method and amylose from cassava starch presented a higher encapsulate bixin content than obtained with Hylon VII figure 1. This was probably due isolated amylose from cassava starch may have a great capacity of film-forming, which entrapped the bixin and gave more protection during the processing. Barbosa et al., (2005) reported that bixin encapsulated with maltodextrin has lower encapsulation efficiency due probably to low film-forming capacity compared to gum Arabic. It known that the quantity of a guest molecule inside of delivery systems is affected by the type of formulation and technique used (De Sousa Lobato et al., 2013). In this study also, the differences in the composition of each formulation and the types of starch used resulted in a bixin content outside of matrix different from these obtained inside of starch matrix. Indeed, starches from different botanical sources differ in their structural characteristics resulting in differences in their properties such as gelatinization, pasting and retrogradation properties (Srichuwong & Jane, 2007). Spada et al., (2012) suggested that carotene on surface degrade more easily during drying process and this increase the losses of carotene. The influence of type of starch on the carotenoid surface content was also reported by Loksuwan (2007).

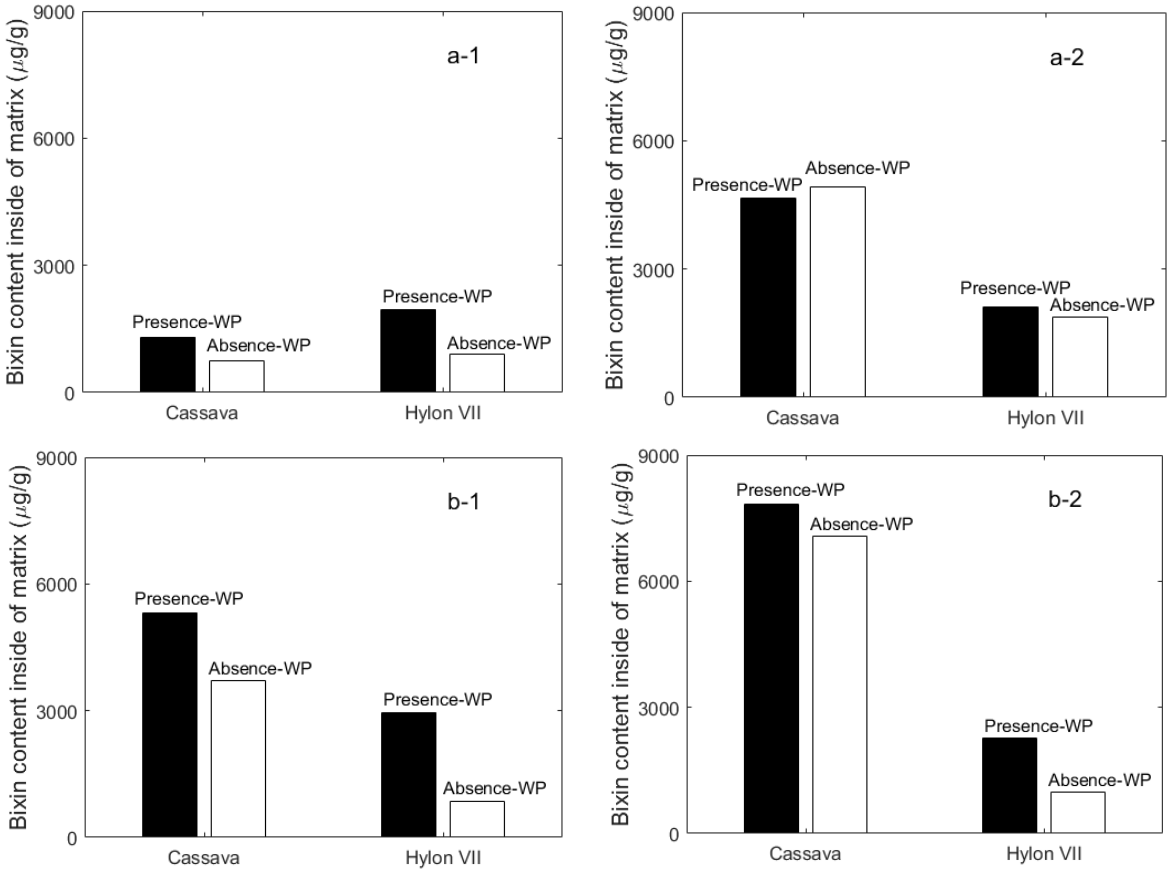


**Figure 1.** Response plot for the effect of whey protein presence and type of starch. 8 % (a) and 2 % (b), at 60 °C (1) and 90 °C (2).

These authors used acid-modified tapioca starch, native tapioca starch, and maltodextrin as wall material in the encapsulation of β-carotene by spray drying, obtaining a lower surface carotene using modified tapioca starch than obtained with native tapioca starch. In the case of microencapsulates β-carotene using native pinhão starch, hydrolyzed pinhão starch and their mixture with gelatin as coating material by freeze-drying. The hydrolyzed pinhão starch showed a lower carotenoids content than observed by native pinhão starch (Spada et al., 2012). Figure 1 illustrated three-dimensional response plots in function of two factors and keeping the other constant for the encapsulate bixin inside of matrix. In the microencapsulation using the alkaline method, the encapsulate bixin content inside of matrix increase with the presence of proteins, regardless of the type of amylose used. Also increases with the increase in the temperature at 8% of amylose (Figure 1, a-1 and a-2). Nevertheless, the encapsulate bixin using 2% of amylose (Figure 1, b-1 and b-2) was higher than obtained with 8% of amylose. This result suggests that probably an increase in the viscosity of starch dispersion during encapsulation process affect the diffusivity of bixin, resulting in a decrease in the encapsulate bixin content.

Table 3 shows the surface and bixin encapsulated obtained by ultrasound treatment. Table 4 shows the coefficients of the model according to equation 4. The analysis of variance (ANOVA)

indicated that the factors were all significant ($p < 0.05$). Interaction terms do not have significant on $L^*$ parameter. Encapsulated bixin ranged between 1234.0 µg /g to 3787.68 µg /g for bixin inside of matrix, and between 267.26 µg /g to 6210.43 µg /g for bixin on the surface. There was a tendency to increase the encapsulate bixin inside of matrix with an increase in the ultrasound power level when it was use 8% of amylose, regardless of treatment time Figure 2. It should be noted the maximum values achieved for this method was higher than obtained with Hylon VII without protein in the alkaline method. The sonication is a method that cause degradation of retrograded starch and chemical modification of starch leading to the reducing of particle size, molar mass and formation of shorter chain (Baxter, Zivanovic, & Weiss, 2005; Montalbo-Lomboy et al., 2010; Zhu, Li, Chen, & Li, 2012). It is probably that this short chain allows more easily the accommodation of the bixin molecules increasing the encapsulate bixin content. Spada et al., (2012) suggests that in the case of encapsulation of β-carotene using hydrolyzed starch, the small molecules of the hydrolysates may better facilitate the packing of the carotene in the matrix. On the other hand, the encapsulate bixin was high at low concentration of Hylon VII, and treatment time 20 min Figure 2a. It seems likely that an increase in the material content might lead a decrease of the encapsulate material. Indeed, Rocha et al., (2012) encapsulate lycopene by spray drying using modified starch obtaining efficiency between entre 21% to 29 % . Shu et al., (2006) encapsulate lycopene in gelatin and sucrose by spray dryer as well, both authors, reported that a high material wall content induces to a lower encapsulate efficiency.

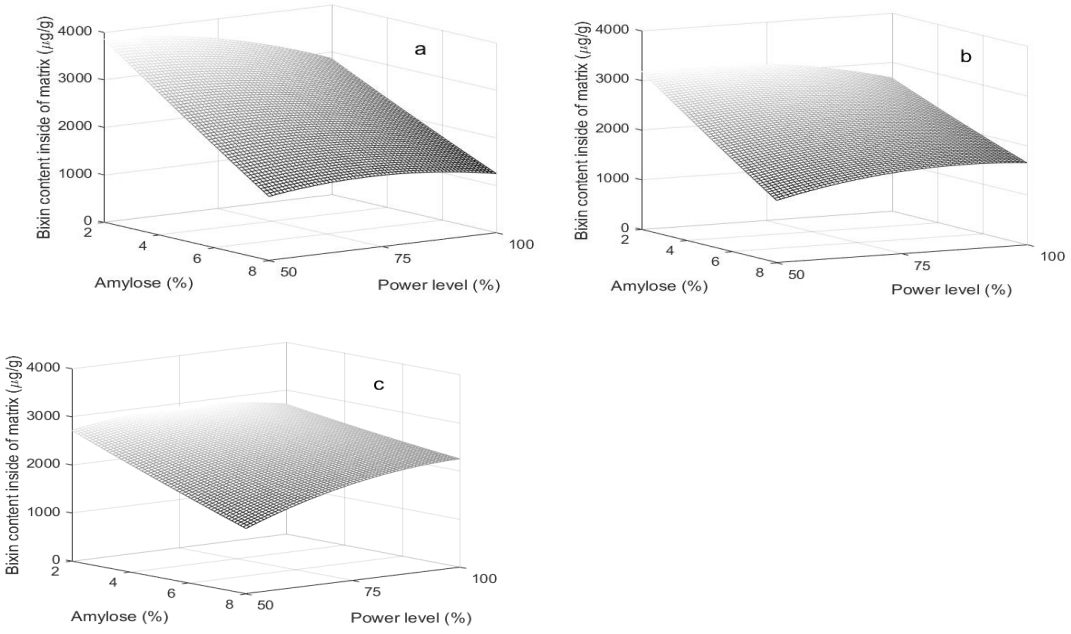


**Figure 2**. Encapsulate bixin inside of starch matrix by ultrasound method. 20 min (a), 30 min (b) and 40 min (c).

**Table 3**. Surface and bixin encapsulation and encapsulation efficiency obtained by ultrasound treatment.

| Run | Factors | | | Bixin content inside of matrix (µg/g) | Bixin content outside of matrix (µg/g) | Total bixin content (µg/g) | Encapsulation efficiency (%) |
|---|---|---|---|---|---|---|---|
| | **A**- Amylose (%) | **B**-Power level (%) | **C**-Time (min) | | | | |
| 1 | 2 | 100 | 40 | 2627.03 | 3913.51 | 6540.54 | 59.83 |
| 2 | 4.4 | 70 | 40 | 2623.44 | 655.17 | 3278.61 | 19.98 |
| 3 | 2 | 82 | 20 | 3467.89 | 5020.18 | 8488.07 | 59.14 |
| 4 | 2 | 50 | 20 | 3787.68 | 6210.43 | 9998.10 | 62.12 |
| 5 | 8 | 70 | 28 | 1676.03 | 430.28 | 2106.28 | 20.43 |
| 6 | 2 | 50 | 33 | 3147.17 | 3147.17 | 6294.34 | 50.00 |
| 7 | 5.5 | 79 | 20 | 2100.03 | 722.03 | 2822.03 | 25.59 |
| 8 | 2 | 50 | 33 | 2900.43 | 2900.42 | 5800.85 | 50.00 |
| 9 | 2 | 82 | 20 | 3460.01 | 4996.39 | 8456.39 | 59.08 |
| 10 | 5.9 | 50 | 20 | 2283.00 | 1632.26 | 3915.26 | 41.69 |
| 11 | 4.4 | 100 | 28 | 2232.09 | 615.90 | 2847.99 | 21.63 |
| 12 | 7.4 | 75 | 38 | 1773.01 | 267.26 | 2040.26 | 13.10 |
| 13 | 8 | 50 | 40 | 1349.04 | 427.01 | 1776.05 | 24.04 |
| 14 | 4.4 | 70 | 40 | 2550.01 | 1501.57 | 4051.57 | 37.06 |
| 15 | 8 | 100 | 20 | 1234.02 | 426.06 | 1660.06 | 25.67 |
| 16 | 8 | 100 | 40 | 2361.56 | 582.15 | 2943.71 | 19.78 |
| 17 | 4.4 | 100 | 28 | 2228.02 | 580.23 | 2808.23 | 20.66 |
| 18 | 8 | 70 | 28 | 1632.54 | 365.78 | 1998.32 | 18.30 |
| 19 | 7.4 | 95 | 30 | 1729.13 | 299.81 | 2028.94 | 14.78 |
| 20 | 5.5 | 51 | 31 | 2007.97 | 1128.29 | 3136.27 | 35.98 |

**Table 4.** Coefficients of the linear, quadratic and interactive terms for the model in the encapsulation by ultrasound.

| | Bixin content inside of matrix (µg/g) | Bixin content outside of matrix (µg/g) | *L** | *a** | *b** | °*hue* |
|---|---|---|---|---|---|---|
| $\beta_0$ | 2378.08 | 578.44 | 44.72 | 32.32 | 38.2 | 48.45 |
| $\beta_A$ | -742.08 | -1677.56 | 4.25 | 0.09 | 4.01 | 3.72 |
| $\beta_B$ | -33.30 | -227.14 | -5.96 | 0.77 | -2.88 | -3.49 |
| $\beta_C$ | -30.37 | -523.05 | 10.01 | -0.26 | 6.76 | 5.67 |
| $\beta_{AB}$ | 224.48 | -118.54 | n.s | 1.2 | 1.05 | n.s |
| $\beta_{AC}$ | 288.90 | 369.10 | n.s | -3.22 | -4.9 | -2.08 |
| $\beta_{BC}$ | 251.22 | 510.35 | n.s | 0.83 | 4.16 | 3.32 |
| $\beta_A^2$ | 23.66 | 1377.12 | n.s | n.s | -2.1 | n.s |
| $\beta_B^2$ | -208.16 | n.s | n.s | n.s | 2.12 | n.s |
| $\beta_C^2$ | 100.15 | 606.51 | n.s | n.s | -2.67 | n.s |
| $R^{2pred}$ | 0.82 | 0.80 | 0.82 | 0.96 | 0.88 | 0.94 |
| $R^{2adj}$ | 0.96 | 0.94 | 0.94 | 0.98 | 0.97 | 0.99 |
| C.V. % | 5.96 | 25.20 | 3.38 | 1.35 | 1.25 | 0.44 |
| Press | 2.06E'6 | 1.34E´7 | 140.7 | 13.35 | 25.06 | 5.38 |
| Modelo (p-valor) | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

n.s: non-significant term.

**Encapsulation efficiency**

Encapsulation efficiency depends on the type of wall material and technology used in the encapsulation. Commonly are used carrier such as starch, pectin, cyclodextrins, modified starch, cellulose, guar gum, whey protein, chitosan, alginate, dextrin as carries in the delivery system preparation (Fathi et al., 2014; Janiszewska-Turak, 2017; Rodea-González et al., 2012). The delivery systems prepared with these wall materials are used as reference to compare the encapsulate bixin and encapsulation efficiency obtained in the current study. Encapsulation efficiency ranged between 13.10% to 62.12% and 17.27%-94.48% by ultrasound treatment and alkaline method. The results are according to obtained for other authors. For instance, De Sousa Lobato (2013) reported encapsulation efficiency of bixin about 98%, in a system prepared by the interfacial deposition of preformed poly-ε-caprolactone. The authors associated the high encapsulation efficiency with the presence of triglycerides in the system, which improved of bixin solubility. Encapsulation efficiency of bixin using Arabic gum and maltodextrin by spray drying ranged between 75% to 86% (Barbosa et al., 2005). De Marco et al., (2013) encapsulates annatto extract by spray drying using a combination of maltodextrin and gum arabic. They reported efficiency about 75.69%. The encapsulation of bixin with poly(3-hydroxybutirate-co-hydroxyvalerate) and dichloromethane as organic solvent using the technology of supercritical carbon dioxide, achieves values between 6.36% to 92.02% (Boschetto et al., 2014). Molecules

similar to bixin has been encapsulated by spray drying of multiple emulsions with encapsulate efficiency of 87%, using gellan and mesquite gum (Rodríguez-Huezo, Pedroza-islas, Prado-Barragán, Beristain, & Vernon-Carter, 2004). Shen & Quek (2014) reported encapsulation efficiency of astaxanthin emulsions ranged between 63% to de 95 %, using as wall material proteins by spray drying. Sharif et al., (2017) reported encapsulation efficiency higher than 90% in the preparation of microcapsule of β-carotene and eugenol by spray drying using modified starch.

Regarding color of samples prepared using ultrasound and alkaline method, Table 2 and 4 shows the coefficient of model for color parameters according to equation 4. The parameter *a** that represent intensity of redness varied between 24.6 to 36.7, and between 25.21 to 39.00 and the parameter *b** ranged between 16.03 to 50.01 and between 31.50 to 44.77 for samples prepared using precipitation from acid solution and ultrasound treatment respectively. The °hue parameter, which represents the color tonality, varied between 32.93 to 59.32 and from 44.92 to 52.6 standing between red (°hue = 0) and yellow (°hue = 90). The °hue is an important parameter since it allows to establish the redness of samples treated and the effect of two methods under the original bixin color. Results indicated that there is a tendency of samples prepared with Hylon VII to be more close orange color those obtained with cassava amylose. Whilst microencapsulate bixin by ultrasound are slightly less reddish than the original color of pure bixin.

**Optimization of bixin encapsulation by RSM**

Ultrasound treatment and alkaline method conditions were optimized for determining of maximum bixin encapsulate content inside of starch matrix. Optimum method conditions were determined for responses with using desirability function. Jafari et al., (2008) suggested that in the encapsulation, the wall material should be a powder with minimum surface compound content and maximum retention inside of the matrix. In this sense, for both methods, the encapsulate bixin inside of starch matrix was maximized keeping the parameter °*hue* between 42 to 52, which represent a color tonality similar to bixin. The optimized conditions for encapsulate bixin by ultrasound are the following: 2% of amylose, 150 W and 20 min of treatment, with an encapsulate bixin content predicted for the model of 3864.1 µg/g. In the case of encapsulation using alkaline method, the optimized conditions were the following: 2 % amylose from mandioca with protein, temperature 68 °C, with an encapsulate bixin content predicted for the model of 5713.98 µg/g.

**Verification of model**

Encapsulation process using the two methods was carried out with the optimized conditions, with the goal of verified the encapsulate bixin predicted by the models. Encapsulate bixin content achieve by ultrasound treatment (2% amylose, 150 W, 20 min) was 3872. 23± 3.2 µg/g. Regarding the alkaline method, the encapsulate bixin content using (2% amylose mandioca with protein, 68 °C) was 5721.98 ± 4.3 µg/g. There were no differences observed between encapsulate bixin predicted and experimental. The models may be used for predicted propose.

## Conclusion

Encapsulation of bixin was carried out by ultrasound treatment and alkaline method. Encapsulate bixin content obtained using both methods were compared using a response surface methodology. RSM results shows that the bixin encapsulate content compounds was influenced by all factor in both methods. Encapsulation using alkaline method and ultrasound could be used successfully to encapsulate bixin with good encapsulation efficiency. However, the bixin encapsulate and encapsulation efficiency achieved by alkaline method is higher than obtained by ultrasound. The optimized condition shows that to improve the encapsulate bixin content inside of matrix is ideal to use cassava amylose with protein. Future studies are desirable to study the effect of the increase of protein concentration under microencapsulation in both methods.

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