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Microencapsulation of Annatto Seed Extract: Stability and Application

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Annatto is the most widely used natural colouring in the food industry, although with a certain degree of difficulty in its applicability due to its lower stability when compared to synthetic colorants. It has as its main component bixin, a substance responsible for its reddish-orange hue and water-insolubility, due to its nonpolar structure. In order to increase stability and the use of natural colorants as food ingredients, this study proposed to encapsulate the annatto seed extract, using maltodextrin and Arabic gum as encapsulating agents by the method of spray drying. The obtained powder was applied to distilled water in order to evaluate the stability, by colorimetry, of the colorant under the influence of light during the storage period. Tests for solubility, water activity and efficiency of microencapsulation were performed to characterize the encapsulated material. The choice of the technique and conditions of microencapsulation was found to be appropriate, given the obtained 75.69 % microencapsulation efficiency and 72 % solubility of the encapsulated material. During the stability test, the final product was subjected to cycles of light and darkness in a eighteen-day period, making it possible to ascertain the deleterious effect of light upon natural colorants, with an observable degradation of the red coloration, and greater bixin stability in the dark.

1. Introduction

The natural colouring annatto is the most widely used colorant in the food industry, representing about 70 % of all natural colouring employed and 50 % of all natural ingredients that perform this function. The annatto contains red pigments obtained from the seed of the annatto (*Bixa orellana, L.*), a plant indigenous to South America and other tropical regions of Central America, Africa and Asia. Theses pigments are extracted from seeds husks and consist mainly of alpha-bixin (lipid-soluble), corresponding to 80% of the total carotenoids present in annatto (Barbosa and Mercadante, 2008). However, carotenoids have a system of conjugated double bonds which, when added to the food matrix undergo oxidation reaction and, consequently, loss of food colouring. This makes them susceptible to high temperatures, light, oxygen availability and low pH, reducing its stability and limiting its use in some food products (Constant et al., 2002).
The growing demand for the use of natural colouring, as a replacement for synthetic colouring, has encouraged the development of new research in the forms of conservation of these pigments, given their high chemical instability (Azeredo, 2005). The most important forms of conservation are pigment microencapsulation and the addition of antioxidants (Valduga et al., 2008).
The microencapsulation technique is defined as the coating of solid particles or droplets of liquid or gaseous materials, forming microcapsules, which release their contents at controlled rates under specific conditions. The microcapsules may have size ranging from fractions of a micron to several millimeters, having different forms, all depending on the materials and methods used in their preparation. The outer

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material, which gives the microcapsule its form, is called encapsulating agent, while the inner ingredient constitutesthe active material (Azeredo, 2005).

The microencapsulation of colorants is intended to protect them against oxidation, providing an increased shelf life, enabling a more efficient solubilization and incorporation in foods (Favaro-Trindade et al., 2008).

There are several techniques that can be used for microencapsulation, the selection of the technique is dependent on the intended application of the microcapsule, its desired size desired, the release mechanism and the physicochemical properties of both the active material and the encapsulating agent (Favaro-Trindade et al., 2008). As examples of techniques employed in the microencapsulation of food ingredients on may mention extrusion, fluidized bed, coacervation, lyophilization, spray drying, spray cooling, molecular inclusion, inclusion in liposomes (Azeredo, 2005);

Spray drying technique is the most commonly used technique in the food industry on account of its cost-effectiveness, easy availability of equipment and low production costs, especially when compared to most other techniques (Barros and Stringheta, 2006). Spray drying involves the atomization of emulsions in a drying medium at high temperature causing a very rapid water evaporation, which in turn results in the formation of a capsule and the trapping of the ingredient inside a coating material (Favaro-Trindade et al., 2008).

One of the main factors influencing the stability of encapsulated compounds is the nature of the encapsulating material. The ideal encapsulating agent must show emulsifying properties, the ability to form films, biodegradability, low viscosity and low hygroscopicity, and must also prove to be low cost. In the practice, it is rare that a single a single encapsulating agent should possess all the properties mentioned, making it common to use a combination of two or more components (Azeredo, 2005).

Arabic gum, the most commonly used encapsulating agent in spray drying, presents compositional characteristics which are seenas ideal for the microencapsulation of lipid contents, as it both serves as an active surface agent and in drying the matrix (Tonon et al., 2010). Maltodextrins have low emulsifying capacity and low viscosity at high concentrations and have been studied as potential substitutes for Arabic gum in atomized emulsions, or used in conjunction with Arabic gum to satisfy the required properties of the encapsulating material (Azeredo, 2005).

Thus, the objective of this study was to encapsulate the annatto extract, using a combination of maltodextrin and Arabic gum as encapsulating agents, applying the spray drying technique, and evaluate the stability of the encapsulated material with application in aqueous solution.

2. Materials and methods

2.1 Materials

Bixin extraction from annatto seeds was performed with commercial ethanol (92.8 %) as solvent, according to the Prentice-Hernandez and Rusig methodology (1992). Annatto seeds were mixed with commercial ethanol, in a ratio of 1:2 (annatto seeds: ethanol), subjecting them to extraction under mechanical stirring for one hour. The extract was sieved, removing the seeds. The recovered extract was placed in a glass container to precipitate, for four hours. The supernatant was discarded and the pigment was subjected to rapid drying, in an forced air circulation oven, at 45 °C, for 30 minutes. The total bixin present in the extract was of 275.1 mg/mL.

Arabic gum (85 % - IMPEX) and maltodextrin DE10 (MOR REX 1910) were provided by Corn Products Brazil. All reagents used were analytical grade.

2.2 Methods

2.2.1 Microencapsulation of natural colouring

Arabic gum and maltodextrin, in the ration of 1:1 (w/w), were hydrated in 200 mL of water, at 60 °C, under stirring. The bixin extract was added to the solution of encapsulating agents, in the ratio of 1:4 (colouring:encapsulant) (w/w), and homogenized in a mechanical stirrer at 1200 rpm for 30 minutes until the temperature reached 30 °C.

The dispersion was subjected to drying in a spray drier - Labmaq Brazil, Model MDS 1.0, with drying temperatures of inlet air of 180 °C and outlet air at 130 °C, nozzle diameter of 0.7 mm, pressure atomizing of 3 Kgf/cm^2, average flow rate of the drying air of 40 L/min flow, and an average feed flow rate of 0.4 L/h (Barbosa and Mercadante, 2008).

The product collected in the form of powder was stored in a glass bottle wrapped in foil and stored in a freezer for further characterization and application.

2.2.2 Extraction and quantification of bixin

The extraction and quantification of the total bixin present in the microcapsules and bixin present only on the surface of the microspheres were determined according to Barbosa et al. (2005). The microencapsulation efficiency (% EM) was calculated according to McNamee et al. (2001) by the equation (1):

$$\%EM = \left(\frac{TB - SB}{TB}\right).100 \tag{1}$$

Where TB is the total bixin present in the microcapsules and SB is the bixin present on the surface of the microspheres.

2.2.3 Solubility test

The solubility test of the microencapsulated material in powder form was performed in triplicate according to the methodology described by Landim (2008). Bixin powder was added to distilled water (1 % w/v), forming a solution. The solution was centrifuged at 3000 rpm for 5 min. The supernatant was placed on Petri plates and carried circulating air oven at 105 °C for 5 h. The solubility was calculated by weight difference.

2.2.4 Water activity

The water activity test was performed on Aw Sprint apparatus- TH 500 – Swiss Made, Novasina.

2.2.5 Effect of light on stability of the extracts

The bixin powder was added to distilled water and stirred. This solution was distributed in test tubes of 10 mL, with screw cap, and stored under fluorescent light (15 W) or in the dark at room temperature of 25 °C ± 1 °C. Samples, in triplicate, were collected for assessment of color in digital colorimeter - Color Reader CR-10 model, Konica Minolta. The system used was the CIEL*a*b*. The pigment degradation was followed for 18 days, a period when the degradation rate is fast.

3. Results and discussion

The microcapsules studied had a microencapsulation efficiency (% EM) of 75.69 %. This result was very close to that reported by Barbosa and Mercadante (2008) who had a microencapsulation efficiency of bixin microencapsulated in maltodextrin and Arabic gum (1:1) of 81 %, using bixin from annatto seeds extracted with ethyl acetate and subjected to a crystallization process in ethanol. The % EM of microencapsulated carotenoid, by spray drying, in gellan and mesquite gum ranged from 72.8 to 87.5 %, in a study of Rodríguez-Huezo et al. (2004).

Maltodextrin and Arabic gum possess properties of high water solubility and emulsifying characteristics. They are excellent encapsulating agents to use in spray drying (Cano-Chauca et al., 2005). Bixin is a lipid-soluble substance, however, in encapsulated form on Arabic gum and maltodextrin, it had a water solubility of 72 %. It can be seen by the study that there was an interaction between the pigment and the encapsulating agents, resulting in a water soluble powder. Landim (2008) obtained a percentage of water solubility of annatto microencapsulated with Arabic gum and maltodextrin, in a ratio of (2:1), of 68 %.

The microcapsules of bixin had low water activity, a value of 0.175 at 25 °C, indicating good stability of the powder.

In light fastness test, there was a decrease in a* and b* coordinates, and an increase in a L* coordinate. The samples stored in the presence of light showed a significant decrease of 22.34 % and 32.4 % in the a* coordinate and b* coordinate, respectively. The L* coordinate had an increase of 18.1 %. Samples stored in the dark showed a reduction of 8.67 % in the a* coordinate, while no change was observed in the b* and L* coordinates. As expected, the light had a deleterious effect on the colouring in aqueous solution, where the red compounds, represented by a* coordinate, and the yellow compounds, represented by b* coordinate, had a rapid degradation in the presence of light, whereas in the dark there was not any significant change in the yellow compounds.

Generally, the color degradation occurs in two distinct periods: during the initial period, the degradation rate is very fast, up to about four to six weeks, and the second period the degradation rate is slow (Rodriguez-Huezo et al, 2004). In this study, the pigment degradation was accompanied for 18 days, namely the first period of degradation, when the coloration is lost rapidly, as seen in Figure 1.

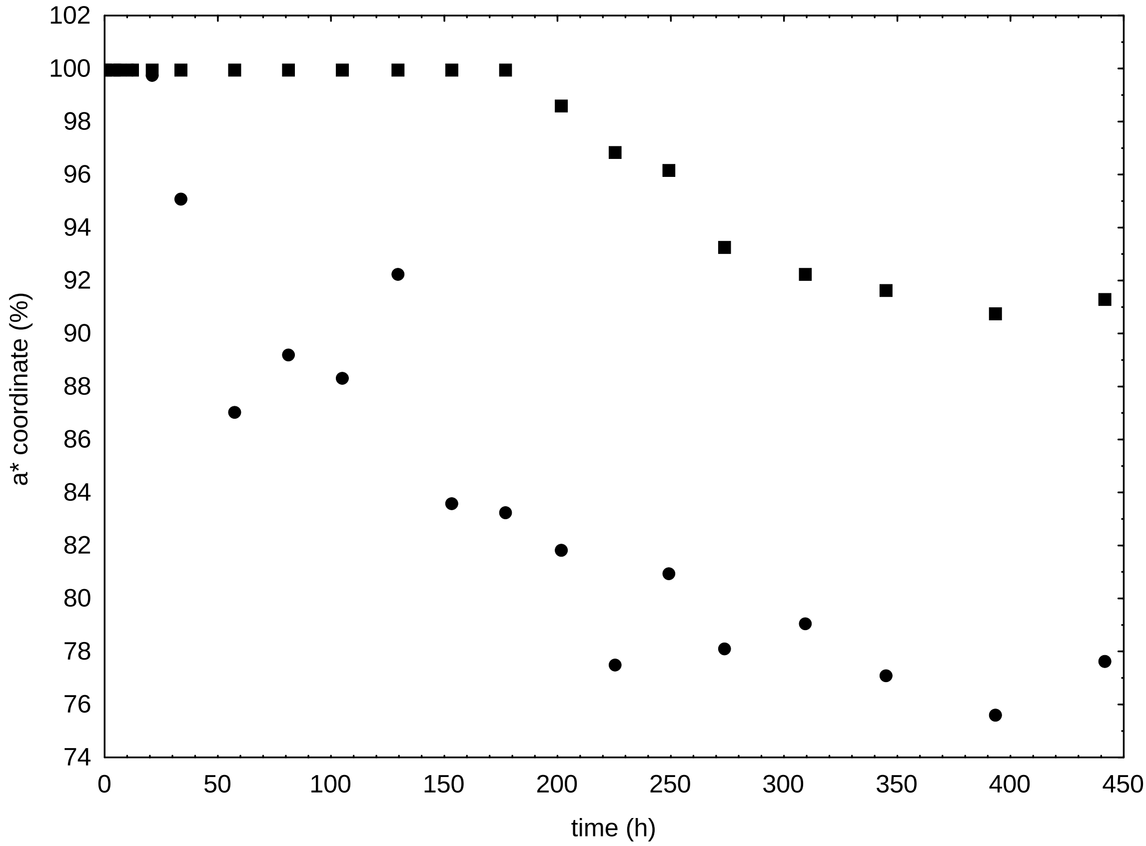

Figure 1. Degradation curves of microencapsulated bixin in aqueous solution, (●) under ilumination (15 W), (■) in dark conditions.

Given the importance of a* chromaticity in the study of the bixin colouring stability in aqueous solution, the degradation kinetics of carotenoid encapsulated in Arabic gum and maltodextrin was evaluated. The model that fitted to the degradation of the pigment was the first order kinetic model, which may be expressed by the equation (2) (Yang et al., 2008):

$$a^* = a^*_0 \,.\exp(-k.t) \quad (2)$$

Where a* is a CIEL*a*b* parameter, a^*_0 is a CIEL*a*b* parameter in the zero time, and k is degradation constant.
The half-life time ($t_{1/2}$) of a first order kinetic model is calculated by equation (3):

$$t_{1/2} = \frac{\ln 2}{k} \quad (3)$$

The degradation constants and half-life times of bixin encapsulated in Arabic gum and maltodextrin under ilumination and dark conditions are presented in Table 1.

Table 1. Degradation constants (k) and half-life times ($t_{1/2}$) for bixin degradation under ilumination (15 W) and in the dark conditions.

Experimental conditions	*$k.10^3$ (days^{-1})*	*$t_{1/2}$ (days)*
Light	15.7	44.15
Dark	3.0	231.05

Mercadante and Barbosa (2008) also adjusted first order model for the degradation of bixin encapsulated in Arabic gum and maltodextrin, in aqueous solution, and observed values of degradation constant of 42.10^{-3} h^{-1} in the presence of light, and $0.45.10^{-3}$ $days^{-3}$ in the dark.
Prentice-Hernández and Rusig (1999) studied the effect of light on the stability of bixin microencapsulated in maltodextrin and an emulsifier, and obtained values of degradation constant of $45.9.10^{-3}$ $days^{-1}$ under ilumination conditions, and $1.0.10^{-3}$ d^{-1} in the dark.
The results obtained by colorimetry showed that light has a negative effect on colouring stability, even in encapsulated form, where it was observed that the degradation of bixin in the dark was five times as stable than when exposed to light.

4. Conclusion

The microencapsulation technique of spray drying proved to be an alternative for the adaptation of natural colouring in the preparation of many food products, as it yields a more easily manipulated powder, with a wider range of applicability than the colouring in extract form.
The use of maltodextrin and Arabic gum as encapsulating agents of bixin, a substance with larger amounts of carotenoids, favoured the formation of microcapsules, as evidenced by the high value obtained in the microencapsulation efficiency.
This present study found a positive interaction between the pigment and the encapsulating materials, as it was possible to obtain a high value for bixin solubility. The aforementioned factor is of great importance since this natural colouring is lipid-soluble, which makes its use difficult in products with large amounts of water.
The deleterious effect of light on the natural colouring was confirmed by the results obtained by colorimetry, in which it was observed that the degradation of bixin in the dark was five times more stable than when exposed to light.

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