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ORIGINAL ARTICLE

Effects of environmental conditions on characteristics of annatto seed by-product

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Nomenclature
A, B, C, K, k_1, k_2, z = models' constants; a_w = water activity; H_{ST} = isosteric heat of sorption (kJ mol^{-1}); L^*, a^*, b^* = color features; M_{eq} = equilibrium moisture content (g of water g of dry matter^{-1}); N = number of data; p = number of constants
P = relative percent error (%); PTFE = polytetrafluoroethylene; R = universal constant of gases (8.314×10^{-3} kJ mol^{-1} K^{-1}); R^2 = coefficient of determination;
SE = standard error; x_m = monolayer moisture content (g of water g of dry matter^{-1}); Y and $\hat{Y}$ are the experimental equilibrium moisture content and the calculated equilibrium moisture content, respectively.

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Abstract

Introduction The processing of annatto seeds yields a low-cost by-product known as annatto seed by-product. Due to its remarkable bixin levels, which is a natural antioxidant, the annatto seed by-product could be used in animal feed supplementation. As a low-moisture product, it is important to know information about hygroscopic behavior, changes during storage, package selection and drying equipment design. *Objectives* The annatto seed by-product's hygroscopic behavior was studied at 25 °C and 35 °C by using saturated salt solutions and the static method. *Methods* During storage with different relative air humidity, the annatto seed by-product's isosteric heat of sorption, bixin content and instrumental color parameters were evaluated. *Results* The isotherms exhibited type III behavior and the Kühn model properly fitted the annatto seed by-product's equilibrium moisture data. Above 75% air relative humidity, agglomeration, darkening and fungal development were observed. The isosteric heat of sorption decreased with an increase in moisture content. *Conclusion* Once the equilibrium was established, bixin losses (approximately 43% of the content) and color changes were more prominent at 35 °C, especially under 68% relative humidity.

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Introduction

The annatto (*Bixa orellana* L.) is a tropical shrub whose seeds are covered by a red carotenoid known as bixin. The natural colorants mostly used by the industries are obtained from bixin (Carvalho, 1999). According to Stringheta & Silva (2008), there are three commercial methods to extract bixin from the seed: by immersion in alkaline solutions (which is used mostly), vegetable oil and organic solvents.

The processing of annatto seeds yields a low-cost by-product that is discarded by the industries. After drying and milling, the annatto seeds by-product (ASB) can be reused as manure for crops or as an animal feed supplement. Bressani *et al.* (1983) reported a high level of proteins, fibers and phosphorus, and Demczuk Jr *et al.* (2010) found significant contents of carotenoid bixin in ASB.

Annatto carotenoids are important owing to their antioxidant properties (Kiokias & Gordon, 2003). Lima *et al.* (2006)

reported the presence of substances with anti-inflammatory activity in the *B. orellana* seeds. A large number of studies have been conducted to describe the influence of carotenoids in animal diet. Carvalho *et al.* (2006) studied egg yolk pigmentation through adding sea sources of carotenoids to the diet of laying hens; Harder *et al.* (2010) reported that the use of annatto increased pigmentation in broiler meats; Utiyama (2001) studied the use of ASB as an alternative feed ingredient for pigs' growth; Queiroz (2006) evaluated the effect of ASB in commercial laying hens as yolk pigmentation.

The hygroscopic equilibrium data of agricultural products, especially those presenting low moisture contents, are of remarkable importance. Therefore, many works have been carried out aiming to expressing the equilibrium moisture content as a function of the relative air humidity in a specific temperature. The usual way to describe the capacity of one food to desorb or absorb water is by obtaining sorption isotherms. Through the use of sorption isotherms, it is possible to propose, optimize and model drying processes, and also predict the growth of microorganisms, product shelf life during storage and choose the correct packaging (Samapundo *et al.*, 2007).

The thermodynamic properties of foods provide information regarding water properties and the energy necessary for sorption to take place. The differential heat of sorption, also known as isosteric heat of sorption, is used as an indicator of the water state that is absorbed by solid particles (Goula *et al.*, 2008). As the isosteric heat of sorption can be expressed as a function of a product equilibrium moisture content, it is a suitable property for estimating the energetic requirements and limits of a drying process (Wang & Brennan, 1991).

Taking into account the importance to know the hygroscopic features of a food and the energetic requirements of a drying process, including materials with compounds that undergo thermal degradation, the aim of this work was to obtain the experimental sorption isotherms for the ASB by using the static method at 25 °C and 35 °C. The suitability of various mathematical models used for fitting the generated data was also investigated, yielding the value of critical moisture content for the bran storage. Instrumental color measurements (CIE $L^*a^*b^*$ scale), chemical composition and bixin content were performed.

Materials and methods

Annatto seed by-product

The ASB was provided by Paschoini Agro (São Sebastião do Paraíso, Minas Gerais, Brazil). Once received, the samples were stored at −18 °C until use, in order to avoid bixin losses. The grinded seeds ($3–75 \times 10^{-3}$ mm diameter) were passed through a set of vibrational sieves ($2.3–75 \times 10^{-3}$ mm) in order to standardize the raw material for the further experiments.

Chemical composition

Moisture content

Moisture content of ASB (g of water 100 g^{-1} of dry solids) was determined by dehydration during 24 h in an oven at 105 °C (Association of Official Agricultural Chemists (AOAC) 2010).

Ash and mineral content

Ash was determined after the sample incineration in a muffle at 550 °C (AOAC, 2010). The minerals' concentrations (Ca, Cu, Fe, K, Mg, Mn, P, Na, Zn) were evaluated by the technique of inductively coupled plasma optical emission spectrometry (AOAC, 2010).

Fat

Total fat content was determined by the Soxhlet method, with petroleum ether as a solvent at 40 °C (AOAC, 2010).

Protein

Protein content was determined by the Kjeldahl procedure using 6.25 as nitrogen conversion factor (AOAC, 2010).

Fiber

The enzymatic and gravimetric protocols were used to determine the dietary fiber content, according to AOAC (2010).

Carbohydrates

Total carbohydrate content was determined by subtracting the sum of moisture, protein, fat, fiber and ash content (%) from 100.

Experimental determination of equilibrium moisture data

The ASB was previously dried by hot air in an oven operating at 40 °C until constant mass was reached.

The method to obtain the experimental data was adapted from the static procedure suggested by Kimura & Maeda (1993), which used plastic containers hermetically closed to form an atmosphere with constant relative humidity. Saturated saline solutions [LiCl, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl, KCl] were used to create the atmospheres with controlled relative humidity ranging from 11% to 84%, according to Kitic *et al.* (1986).

Three samples of ASB (10 g and 5 mm of thickness) were placed inside aluminum vessels presenting a diameter of 40 mm. The vessels were placed inside plastic containers (170 mm × 170 mm × 120 mm) with saturated saline solutions, which were closed and placed in an oven operating at 25 ± 1 °C or 35 ± 1 °C. The samples' weight was monitored until the gravimetric equilibrium was reached.

In order to estimate the experimental error, the assay was repeated under the same conditions.

Analysis of equilibrium moisture data

The ASB hygroscopic behavior was predicted by fitting the experimental data with theoretical isotherms equations (Table 1), proposed by various authors (Lewicki, 2008).

In order to evaluate the quality of the fit provided by each mathematical model, a nonlinear regression analysis was performed (Gauss–Newton method) by using the Statistica 7.0 software (Statsoft, Tulsa, OK, USA). The suitability of each model to represent the experimental data was analyzed on the basis of the determination coefficient value (R^2), the relative percent error value (P) and standard error (SE), calculated as shown below:

$$P(\%) = \frac{100}{N}\sum\left(\frac{\left|Y-\hat{Y}\right|}{Y}\right) \quad (1)$$

$$\mathrm{SE} = \left[\frac{\sum\left(Y-\hat{Y}\right)}{N-p}\right]^{0.5} \quad (2)$$

Table 1 Theoretical models used to fit the moisture sorption isotherms

Model	Equation
BET (Brunauer–Emmett–Teller)	$M_{eq} = x_m C a_w / \{[1 - a_w][1 + (C - 1)a_w]\}$
GAB (Guggenheim–Anderson–de Boer)	$M_{eq} = (x_m C K a_w)/[(1 - K a_w)(1 - K a_w + C K a_w)]$
Lewicki	$M_{eq} = A[(1/a_w) - 1]^{B-1}$
Kühn	$M_{eq} = K(1/a_w)^{-z} - B$
Kühn simplified	$M_{eq} = (k_1/\ln a_w) + k_2$

M_{eq}, equilibrium moisture content (g of water g of dry matter^{-1}); x_m, monolayer moisture content (g of water g of dry matter^{-1}); a_w, water activity; A, B, C, K, k_1, k_2, z, constants of models.

where Y and $\hat{Y}$ are the experimental equilibrium moisture content and the calculated equilibrium moisture content, respectively. N is the number of data and p is the number of constants in the model. A model can be considered acceptable when it presents the relative percent error minor than 10% ($P < 10\%$), higher values of R^2 and lower values of SE (Lewicki, 2008).

Isosteric heat of sorption calculation

The isosteric heat of sorption (H_{ST}), defined by Samapundo *et al.* (2007) as the total heat of water sorption minus the heat of water vaporization, was calculated by using the integrated form of the Clausius–Clapeyron equation:

$$H_{ST} = (RT_1T_2)/(T_2 - T_1)\ln(a_{w2}/a_{w1}) \quad (3)$$

where R is the universal constant of gases (8.314×10^{-3} kJ mol^{-1} K^{-1}) and a_{w2} and a_{w1} are the water activities at the temperatures T_2 (35 °C) and T_1 (25 °C), respectively.

Bixin content measurement by high-performance liquid chromatography

The extraction of bixin from the ASB was performed at each relative humidity point, in triplicate, according to the procedure described by Tocchini & Mercadante (2001). Before the chromatographic analysis, the samples were previously filtered in polytetrafluoroethylene membranes with 0.22 μm of diameter.

A Shimadzu high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) controlled by the Class-VP software and equipped with a Rheodyne manual injector (Phenomenex, Torrance, CA, USA) was used. A Synergi column (3.9 × 150 mm, 4 μm; Phenomenex), a LC-10AD pump (Phenomenex) and a SPD-10A UV-VIS detector (Phenomenex) were used. Other assay conditions were injection volume of 20 μL, wavelength of 470 nm and flow rate of 1 mL min^{-1}.

The chromatographic grade solvents, namely acetonitrile : acetic acid 2% (65:35), were previously filtered in a 0.45-μm membrane before use (Tocchini & Mercadante, 2001).

The bixin quantification was performed by external standard from the analytic curve, which was built after injection of the standard solution under five different concentrations. The selected concentrations were defined on the basis of the ranges expected for each sample. The bixin standard (99.8% purity) used for the analysis was obtained following the method of Rios *et al.* (2007).

Instrumental color measurements

The ASB color features were measured by the MiniScan XE Plus (Hunter Lab, Reston, VA, USA) reflectance spectropho-

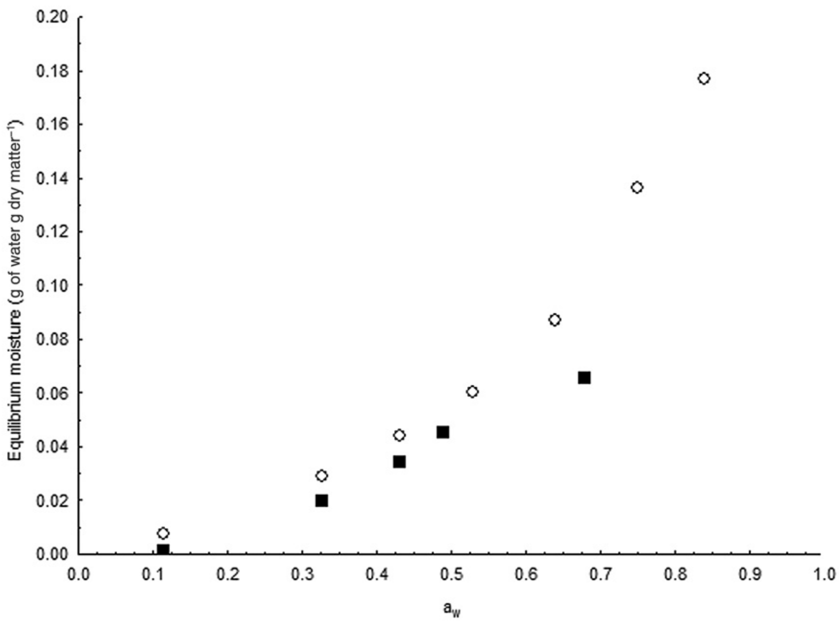

Figure 1 Equilibrium moisture data for annatto seed by-product at 25 °C (○) and 35°C (■) for different relative humidity of air.

tometer. The D65 illuminant and the 10° angle for the observer were used. The instrument was previously standardized by measuring the black color glass and a white tile ($X = 78.9$, $Y = 83.9$, $Z = 88.9$). The CIE $L^*a^*b^*$ scale was used to express the measured color (CIE, 1986). The L^* axis represents the lightness, which ranges from 0 (black) to 100 (white). The parameter a^* represents the greenness/redness ($-a^*/+a^*$) and the parameter b^* represents the blueness/yellowness ($-b^*/+b^*$).

Statistical analysis

The obtained results were statistically evaluated by the software MSTAT-C version 2.10 (Michigan State University, East Lansing, MI, USA). The means and the standard deviations were calculated. Analysis of variance and the Tukey's test of means were performed.

Results and discussion

The granulometric distribution assay performed by the ASB showed that most of the particles retained in the sieves measured between 850 and 425.10^{-3} mm. Therefore, particles within such granulometric range were chosen for the further experiments.

Fiber was the main component (42 g 100 g^{-1}) found in ASB. Other components included carbohydrates, 27.21 g 100 g^{-1}; protein, 11.94 g 100 g^{-1} ± 0.13; ashes, 5.65 g 100 g^{-1} ± 0.21 and fat 1.84 mg 100 g^{-1} ± 0.04. The results showed that ASB is a good source of minerals: calcium, 194.03 mg 100 g^{-1} ± 1.68; copper, 1.56 mg 100 g^{-1} ± 0.11; iron, 5.97 mg 100 g^{-1} ± 0.57; phosphorus 336.85 mg 100 g^{-1} ± 4.71; magnesium 304.79 mg 100 g^{-1} ± 1.22; manganese, 9.35 mg 100 g^{-1} ± 0.30; potassium, 2285.12 mg 100 g^{-1} ± 23.02; sodium, 7.87 mg 100 g^{-1} ± 1.94 and zinc, 28.06 mg 100 g^{-1} ± 0.57.

The ASB initial moisture content was 1.14 g 100 g^{-1}. The gravimetric equilibrium moisture was reached after 53 and 28 days when the experiments were carried out at 25 °C and 35 °C, respectively.

The equilibrium moisture data for the ASB as obtained at 25 °C and 35 °C are shown in Figure 1.

The experimental data suggest that, at a constant water activity, the equilibrium moisture content decreased with an increase in temperature. Such behavior can be attributed to a reduction in the number of active sites available for binding with water, resulting the physical-chemical changes in the food system that are induced by an increase in temperature (Goula *et al.*, 2008).

Lima *et al.* (2000) found an equilibrium moisture value of 13.3 g 100 g^{-1} for the whole annatto seed stored at 25 °C under a 0.86 water activity. In the present work, it was found 17.7 g 100 g^{-1} equilibrium moisture at 25 °C under a 0.84 water activity. Such difference could be attributed to the lower particle size of the ASB when compared with the whole annatto seed. Chinnan & Beuchat (1985) observed a

Table 2 Calculated parameters, R^2, P (%) and SE for the fitting the ASB equilibrium moisture data with selected mathematical models at two temperatures

Model	Parameter	R^2	P (%)	SE	Residual plot pattern
	25 °C				
BET	$x_m = 0.383$	0.981	0.124	8.084×10^{-3}	Systematic
	$C = 0.245$				
GAB	$x_m = 2.932$	0.992	0.050	5.283×10^{-3}	Random
	$C = 0.033$				
	$K = 0.582$				
Lewicki	$A = 0.057$	0.980	0.127	8.2×10^{-3}	Systematic
	$B = 0.299$				
Kühn	$k = 0.256$	0.991	0.060	5.798×10^{-3}	Random
	$z = 2.531$				
	$B = -0.010$				
Kühn simplified	$k_1 = -0.031$	0.953	0.308	12.74×10^{-3}	Systematic
	$k_2 = 0.006$				
	35 °C				
BET	$x_m = 0.074$	0.980	0.015	3.385×10^{-3}	Systematic
	$C = 0.650$				
GAB	$x_m = 3.517$	0.984	0.011	3.106×10^{-3}	Systematic
	$C = 0.020$				
	$K = 0.581$				
Lewicki	$A = 0.039$	0.978	0.016	3.520×10^{-3}	Systematic
	$B = 0.079$				
Kühn	$k = 0.140$	0.997	0.002	1.405×10^{-3}	Random
	$z = 1.586$				
	$B = 0.004$				
Kühn simplified	$k_1 = -0.036$	0.984	0.012	3.034×10^{-3}	Systematic
	$k_2 = -0.013$				

ASB, annatto seed by-product; BET, Brunauer–Emmett–Teller; GAB, Guggenheim–Anderson–de Boer; SE, standard error.

similar behavior while comparing the entire cowpeas and their flour.

For both working temperatures, it was visually observed that the samples stored at high relative humidity (above 64%) underwent agglomeration and darkening. The samples stored at 35 °C (75% and 84% relative humidity) also presented microbial spoilage, being discarded before reaching gravimetric equilibrium.

Table 2 shows the calculated parameters of different models used to fit the moisture data of the ASB under different relative humidity and temperature.

All selected models showed a good fit (P lower than 10% and ranging from 0.002 to 0.308). The values of R^2 ranged from 0.953 to 0.997 and SE from 1.405×10^{-3} to 12.74×10^{-3}. Kühn model gave the best fit to experimental data.

In the present work, the Guggenheim–Anderson–de Boer (GAB) model was found to best represent the equilibrium data at 25 °C. According to Timmermann *et al.* (2001), the GAB model was recommended as the fundamental equation for representing the water sorption in food by the European Project Group COST 90 on Physical Properties of Food. Such recommendation was made on the basis of the capacity of the GAB model of representing properly experimental data in a key range of water activity in food (between 0.1 and 0.9). The same authors state that the GAB constant k determines the profile of the isotherm at the higher water activity range, regulating the upswing after medium water activity range. Higher values of k determine a significant upswing.

The monolayer moisture content (x_m) seemed to be more appropriately predicted by the GAB model (Table 2). Such conclusion was made taking into account the values of R^2 and the consideration made by Salwin (1963), who stated that the Brunauer–Emmett–Teller model is not appropriate for predicting the monolayer moisture content for Brunauer type III isotherms (Lewicki, 2008), which is the ASB's case. Type III behavior is characterized by significant moisture uptake only at higher water vapor pressures or at high relative humidity on a given temperature isotherm. This classification was chosen based on the shape of obtained data and in the modified classification proposed by Blahovec & Yanniotis (2009), based on a_w/M_{eq} versus a_w plot.

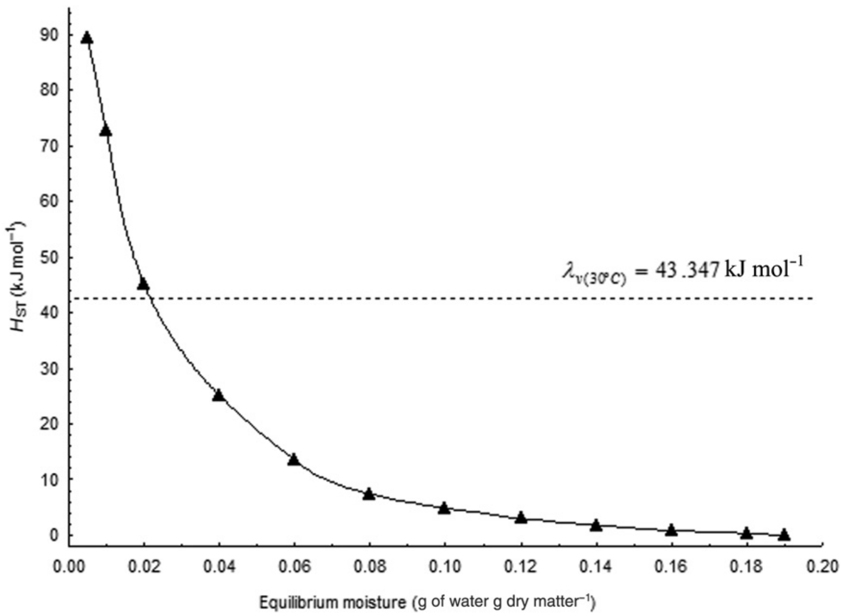

Figure 2 Effect of equilibrium moisture content on the isosteric heat of sorption for the annatto seed by-product. Note: λV = water latent heat of vaporization.

The GAB parameters (Table 2) for Brunauer type III isotherm are in accordance with Blahovec (2004) ($0 < k < 1$ and $0 \leq C \leq 2$). These observations were also found in this study. After analyzing 115 isotherms in literature, Blahovec & Yanniotis (2009) found that only one could be classified as type III. This behavior is common in foods with high carbohydrate content, especially amorphous, which adsorbs water in low contents at lower relative humidity and it absorbs higher water contents with the constant increase in relative humidity.

Figure 2 shows the isosteric heat of sorption values (H_{ST}) as a function of the equilibrium moisture at 30 °C. An increase in the isosteric heat of sorption values occurred with a decrease in the moisture content. At high-equilibrium moisture contents (0.19 g of water g dry matter^{-1}), the isosteric heat of sorption values remained constant and close to zero. Thus, the total heat of sorption corresponds to the water vaporization heat, which suggests the presence of free water under these equilibrium moisture contents.

Goula *et al.* (2008) stated that the fast rise in the isosteric heat of sorption values under low moisture contents is due to the fact that, at initial stages of sorption, there are highly polar active sites on the food surface that are covered by the water monolayer. When the moisture content is below the monolayer moisture content, it is necessary to provide huge amounts of energy for the sorption to take place. With an increase in moisture, the less active sites are covered by water and the formation of multilayers causes a decrease in the isosteric heat of sorption (Pérez-Alonso *et al.*, 2006).

As shown in Figure 2, the change in the slope of the curve coincides with the value of the monolayer moisture content (2.93 g of water 100 g dry matter^{-1}). In this study, it was not found a single observation, which presents similar data in annatto seeds. Although, other works dealing with seeds and their flours show that there is a difference between their isosteric heat sorption values. Samapundo *et al.* (2007) found an isosteric heat of sorption of 55 kJ mol^{-1} at 27.5 °C and 0.07 g of water g of dry matter^{-1} for whole corn grains, while Labuza *et al.* (1985) reported an isosteric heat of sorption of 18.6 kJ mol^{-1} for corn flour.

Figure 3 shows the average bixin concentrations in the ASB obtained by high-performance liquid chromatography analysis after storage at 25 °C or 35 °C.

The initial sample presented bixin levels around 498.81 mg 100 g^{-1}. The ASB had not been evaluated for its bixin content yet. Although, the bixin levels detected in this study are higher than those detected in Brazilian 'colorífico' by Tocchini & Mercadante (2001), (154–354 mg 100 g^{-1}), which is a mixture of annatto and corn bran consumed as seasoning in Brazil.

At 25 °C and 11% of relative humidity, the bixin levels were the lowest among the observed values at this temperature. Such behavior might be related to a higher exposition of bixin to oxygen, causing oxidation. Without the protective

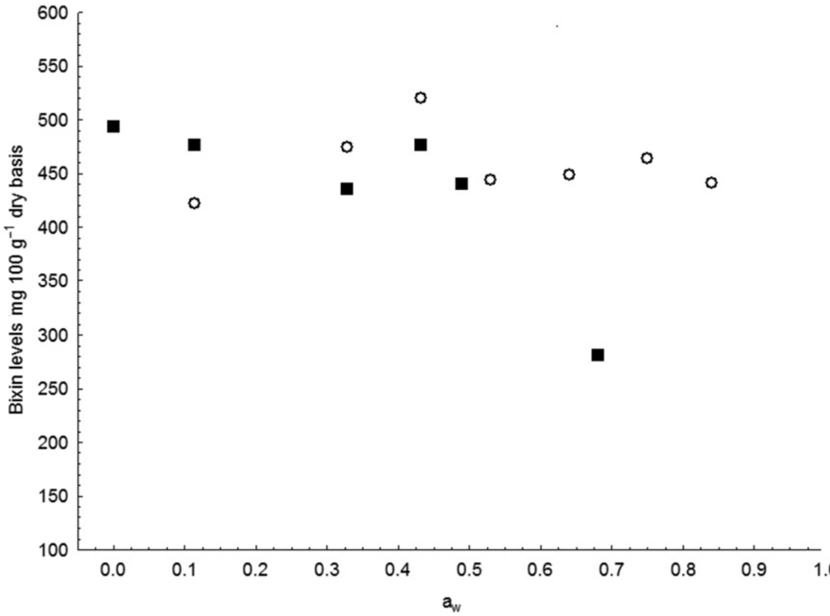

Figure 3 Annatto seed by-product bixin concentration as a function of water activity at 25 °C (○) and 35 °C (■).

Table 3 Annatto seed by-product CIE *L*a*b** parameters after storage at 25 °C and 35 °C under different relative humidity

	Color parameters					
	*L**		*a**		*b**	
a_w	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C
Initial	32.4[a] ± 0.5	32.4[a] ± 0.5	23.8[a] ± 0.3	23.8[a] ± 0.3	29.2[a] ± 0.2	29.2[a] ± 0.2
0.11	32.5[a] ± 0.4	32.4[a] ± 0.8	22.9[b] ± 0.2	23.2[a] ± 0.5	27.7[b] ± 0.6	27.9[ab] ± 1.1
0.32	32.6[a] ± 0.8	32.7[a] ± 0.4	23.0[b] ± 0.4	23.3[a] ± 0.4	27.6[b] ± 0.5	27.8[b] ± 0.3
0.43	32.3[a] ± 0.3	32.5[a] ± 0.3	23.0[b] ± 0.5	23.4[a] ± 0.2	27.4[b] ± 0.4	28.1[ab] ± 0.7
0.52	31.7[a] ± 0.7	32.2[a] ± 0.4	22.9[b] ± 0.7	23.4[a] ± 0.3	27.2[b] ± 0.8	27.7[b] ± 0.9
0.64	28.4[c] ± 1.9	28.9[b] ± 0.7	20.8[c] ± 0.5	17.6[b] ± 2.0	24.2[d] ± 0.8	21.8[c] ± 1.4
0.75	29.9[b] ± 0.3	–	23.1[b] ± 0.2	–	27.0[b] ± 0.1	–
0.84	29.7[b] ± 0.1	–	22.6[b] ± 0.2	–	25.9[c] ± 0.5	–

Different letters in the same column denote statistically significant difference at 5% confidence level.

effect of water at low relative water activity, the lipids degradation is higher.

While at 25 °C, the bixin levels remained roughly constant throughout the storage; at 35 °C, it was observed a decrease in bixin concentration with an increase on water activity, especially at 0.68.

According to Belitz *et al.* (2008), an increase in water activity causes the metals contained in food to move freely and possibly work as catalyzers for lipids and carotenoids oxidation. On the other hand, under high water activities, the metals are diluted in the food matrix, being unable to work as catalyzers. A similar behavior was observed by Prado Filho (1994) when studying the effect of water activity on nut bran lipids oxidation. In addition, at 0.68 a_w, the difference between bixin levels at 25 °C and 35 °C could be explained by the influence of temperature on rate of carotenoid degradation.

Table 3 shows the color features of the ASB as measured after storage at 25 °C and 35 °C.

After evaluating the initial parameters (CIE *L*a*b**), it can be concluded that the ASB sample presented dark-orange color. For both storage temperatures, a decrease in lightness (*L**) and in redness (*a**) was observed with an increase in water activity, being this behavior more prominent at 35 °C. When seen together, the values of CIE *L*a*b** parameters show that the ASB darkened with an increase in storage relative humidity. At 25 °C, the *L** remained constant until a water activity of 0.52 was reached. On the other hand, the *a** and b^* values were affected by storage even at low water activities values. Furthermore, at 35 °C, the L^* and the a^* values were not affected until a water activity of 0.52 was reached. Although, the highest change in color was observed at a 0.64 a_w for experimental conditions.

The darkening and the decrease in redness detected at $a_w = 0.68$ and 35 °C was higher than those observed at 25 °C, even at higher water activities. It can be explained by the dilution of oxidant components in the bran, and this behavior is supported by the bixin degradation under similar conditions.

Conclusions

The experimental conditions evaluated in this study were adequate for elucidating the ASB hygroscopic behavior. The experimental data showed a decrease in the equilibrium moisture content with an increase in temperature under constant water activity. The ASB moisture sorption isotherm obtained was well represented by the Kühn model and Brunauer type III isotherm. An increase in the isosteric heat of sorption occurred with a decrease in the moisture content. The ASBs stored until 68% relative humidity and 35 °C have kept its color and bixin levels.

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