Advance Journal of Food Science and Technology 14(6): 155-165, 2018
DOI:10.19026/ajfst.14.5977
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: November 26, 2017 Accepted: June 19, 2018 Published: October 25, 2018

Research Article

Vacuum Drying of Annatto Seeds (*Bixaorellana*): Drying Kinetics, Optimization and Stability Study

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Abstract: The aim of this work was to study the vacuum drying on some quality properties of annatto seeds (*Bixaorellana*. Quality properties of the dried product were analyzed using the Response Surface Methodology (RSM) considering the vacuum pressure and temperature of the drying process. In addition, the drying kinetics and stability of the dried product were studied. The analysis of the results indicated that Page's model was the most suitable to describe the drying behavior, where the bulk density of the final product and total color change between fresh annatto seeds and dry seeds were not statistically significant. The optimum vacuum drying conditions were 45°C, 10.45 mbar and 12 h, with values of 0.358 for the water activity, 7.588 %(w.b) for the moisture content and 9.61% for the bixin content. Additionally, the bixin content presents a decreasing trend during storage for both conditions of light and darkness, where during 28 days of storage, the loss of bixin was 5.27% for light and 4.14% for darkness.

Keywords: Bixin, chemical characterization, stability, vacuum drying

INTRODUCTION

Nowadays consumer's preferences are based on natural products in the search for a healthy lifestyle. Natural dyes have been generating interest in industries, especially with edible purposes, due to reports of allergies, insomnia, kidney disease, anemia and others illnesses caused by the consumption of synthetic dyes present in food matrices (Albuquerque and Meireles, 2011).

Annatto is a natural dye that can be red to orange depending on the concentration of the solution. The main component is cis-bixin (oil soluble), a resinous coating outside the seed present in a triangular capsular fruit and it is considered to be 80% of the total dye content. This carotenoid is also known in the industry with CAS number E-160B and more commonly as Natural Orange 4, bija and urucum (Giridhar *et al*., 2014; Souza *et al*., 2016).

The main structure of cis-bixin is methyl hydrogen 9′-cis-6,6′-diapocarotene-6,6′-dioate. The double bond in the structure is responsible for the red color of bixin, but at the same time, this confers poor stability during storage as well as sensitivity to oxygen, temperature and light, causing large losses of pigment and economic profits (Barbosa *et al*., 2005; Lobato *et al*., 2013).

Traditional drying techniques are applied for seeds, where forced air convection drying is the most common (Da Costa-Santos *et al*., 2013; Santos *et al*., 2012). Taking into account that the price of annatto seeds usually depends on the bixin content, is important to study new techniques of drying that allow higher concentration values of the carotenoid to be obtained, while avoiding factors that affect the quality of the seeds, in order to obtain a better seller price. Peru is the most important producer of annatto seeds in the world and their seeds are considered to have the best quality, with a bixin content of approximately 3%, while Brazilian seeds, the third largest worldwide producer, only reach a bixin content of 2.7% (Albuquerque and Meireles, 2011).

Dehydration is a usual technique around the world to preserve food and agricultural products. The phenomenon involves heat, mass and momentum transfer, with the purpose of removing moisture from the food matrix (De Bonis and Ruocco, 2008). Vacuum drying is one of the most novel dehydration techniques because of the lower temperatures used during the process by reducing the pressure and oxygen presence. This allows the chemical reactions in the product to be reduced, thus avoiding undesirable quality effects such as the loss of color, aroma and compounds of interest that are sensitive to high temperatures (Da Silva *et al*., 2016; Šumić *et al*., 2016) and it is a novel way to preserve bioactive compounds such as bixin, which is the most important compound in annatto seeds.

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There are two parameters that affect the drying behavior in vacuum drying, the pressure and temperature. Inside the chamber, dehydration takes place at sub-atmospheric pressure, thus reducing the boiling point; the plate provides latent heat to the product allowing the evaporation of water. Furthermore, the temperature of the plate is sustained to be higher than the boiling point of water, thus permitting complete dehydration (Das, 2005).

The use of vacuum as a drying technique has not been used for annatto (*Bixaorellana*) seeds according to the literature and for the first time, the authors are reporting a study relating vacuum drying, kinetics modelling, drying optimization and stability under storage conditions. The purposes of this research work were as follows:

- Study the relationships between the temperature and pressure as well as their influence on the drying kinetics of annatto seeds
- Find a suitable mathematical model for the vacuum drying of annatto seeds
- Determine the optimal vacuum drying conditions that promote the concentration of bixin on dry seeds
- Evaluate the stability of dry seeds for different illumination conditions.

MATERIALS AND METHODS

Raw material: Fresh annatto seeds were collected from the Department of Chocó (Colombia-South America). To make sure that all of the seeds had the same start and to prevent deterioration during experiments, the samples in their natural capsules were packaged and ultra-frozen at -25°C±0.1°C in the facilities of the Agricultural Process Laboratory in the Universidad Nacional de Colombia.

Preparation of the sample: Two hours before the drying experiments, annatto capsules were taken out of the freezer in order to reach ambient temperature (20°C). The seeds were manually extracted and placed in a petri dish in a thin layer of 10 g±0.25 g.

Vacuum drying: Drying experiments were performed in a Memmert VO 200 vacuum dryer. The dryer consisted of a chamber containing a metallic plate, which is heated by an electrical resistance heater. A pump provides vacuum inside the chamber and the vacuometer shows the pressure value. A digital display allows the control of the temperature and pressure. The samples were placed on the plate inside the chamber previously preheated. For drying kinetics, the conditions of the experiment were taken at different pressures (10 mbar and 50 mbar) and temperatures (40°C and 45°C) during twelve hours of the process. The weight loss was verified hourly and performed in triplicate. These parameters were defined by preliminary experiments. This carotenoid is sensitive to high temperatures (T>50°C), thus promoting bixin degradation (Rivera-Madrid *et al*., 2016; Taham *et al*., 2015) and temperatures below 40°C require a prolonged time to reach the equilibrium point (Santos *et al*., 2012), which is not a commercial moisture content, as preliminary studies showed. Šumić *et al*. (2016) report that higher pressures present higher final moisture contents. Since this is the first study using vacuum drying on annatto seeds, the pressure was determined through previous experiments, starting from the selected temperatures and evaluating the equilibrium moisture content within the commercial requirements. The time was a selected based on the period necessary to reach the equilibrium point. Conditions of the experiment were taken based on preliminary studies at different pressures (10 mbar, 30 mbar and 50 mbar), temperatures (40°C, 42.5°C and 45°C) and times (6, 9 and 12 h).

Moisture content: The Moisture Content (MC) was measured in a 108 Memmert oven and the samples were dried, verifying weight loss at 105°C until reaching a constant weight following the (AOAC, 1990) 930.15/90 standard method methodology. The samples used in this test were kept in constant conditions of relative humidity and temperature to guarantee the homogeneity of the initial moisture content. Tests were performed in triplicate for every run for statistical purposes and the moisture content was expressed as a wet basis (w.b.).

Water activity: The water activity was measured in triplicate by a dew point hygrometer at 25°C (Aqualab 3TE series, Decagon, Devices, Pullman, WA, USA). Dry seeds were deposited on sample holders, approximately 1.5 g and the results were recorded after stabilization of the measurement.

Bulk density: This parameter was measured using Colombian Technical Standard NTC 4607/1999, where a container with a known volume is filled with the sample and afterward, the seeds in the container are weighed. The bulk density under free flow conditions is expressed as g/L.

Total color change: The total color change was determined using a Minolta CR-400 Chroma Meter (Minolta Co., Ltd., Osaka, Japan), with illuminant D65 and a 10° observer for reference. From the reflection spectra, color coordinates CIE L*, a* and b* were obtained, where L* indicates the luminosity, a* indicates the green (-) to red (+) chromaticity and b* indicates the blue (-) to yellow (+) chromaticity. Finally, the total color change between fresh annatto seeds and dry seeds was measured using Eq. (1) (Satyanarayana *et al*., 2010). A standard was used to

Table 1: Mathematical models applied in the vacuum drying process

Model name	Model equation	Reference
Lewis	$MR = \exp(-kt)$	(Kaleta and Górnicki, 2010)
Henderson and Pabis	$MR = a\exp(-kt)$	(Diamante *et al*., 2010)
Wang and Singh	$MR = 1 + a * t + b * t^2$	(Kaleta and Górnicki, 2010)
Peleg	$MR = \frac{1-t}{(a+bt)}$	(Mercali *et al*., 2010)
Page	$MR = \exp(-kt^n)$	(Diamante *et al*., 2010)

calibrate the colorimeter. All measurements were acquired three times for statistical proposes.

$$\Delta E = \sqrt{(L_0{}^* - L^*)^2 + (a_0{}^* - L^*)^2 + (b_0{}^* - L^*)^2} \quad (1)$$

Bixin content: The bixin content of the seeds was measured using a Genesys 10s UV-Vis spectrophotometer (Thermo Scientific) following Peruvian Technical Standard NTP 209.256:1991 for every run. The test was realized in triplicate for statistical purposes.

Lyophilization: Lyophilization was performed in a Labconco® lyophilizer at -50°C and 0.140 mbar pressure for 36 h using individual borosilicate drying chambers under controlled ambient temperature (20°C±2) conditions. In total, 30 g of sample was dried in 3 vessels of 10 g.

Mathematical modeling: Several empirical and theoretical mathematical models have been used to describe the drying behavior of foods including seeds and grains (Rabha *et al*., 2017). These models are also known as thin layer models. In this study, the thin layer models shown in Table 1 were used. In these models a, b, k and n are constants. The moisture ratio in thin layer models (MR) is defined as (El-Sebaii and Shalaby, 2013):

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (2)$$

where,

MR = Dimensionless moisture ratio (dimensionless)
M_0 = Initial moisture of the product (kg water/kg product)
M_e = Equilibrium moisture content (kg water/kg product)
M_t = Instantaneous moisture content on a wet basis (kg water/kg product)

Experimental results were plotted for each combination. Models were fitted by non-linear regression analysis performed in DataFit 9. The coefficient of determination (R^2), reduced chi-squared (χ^2) and Root Mean Square Error (RMSE) were used as selection parameters for the mathematical model. The criteria employed to determine the quality of the fit were higher values of R^2 and lower values of χ^2 and RMSE. The χ^2 and RMSE were calculated using equations reported by Vijayan *et al*. (2016).

Table 2: Independent variables and their ranges used in Box-Behnken design

	Coded level		
Level	-1	0	1
Temperature (°C)	40	42.5	45
Pressure (mbar)	10	30	50
Time (h)	6	9	12

Experimental design: For this study, the Box-Behnken experimental design given in Table 2 was used, combined with Response Surface Methodology (RSM), as a system for data analysis, using STATGRAPHICS Centurion XVI.II software. A total of 15 experiments were carried out and the response variables were measured in triplicate to confirm the effect of the independent variable on the responses. Response variables were fitted with the second order polynomial model given in Eq. (3), describing the interaction between the independent variables (pressure, temperature and time) and response variables:

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii} X_i{}^2 + \sum_{i<j=1}^{k}\beta_{ij} X_i X_j + \varepsilon \quad (3)$$

where,

Y = The response
β0 = The interception coefficient
βi and βj = The linear coefficients of regression for the intercept
βii = The quadratic coefficient of regression for the intercept
βij = The interaction coefficient for the intercept
k = The number of independent parameters

In this case 3 and ε is the error. The results were analyzed using an Analysis of Variance (ANOVA) to evaluate the suitability of the model and determine the regression coefficients with a 95% confidence level. Three-dimensional plots were generated to evaluate the influence of significant interactions with the variable responses. The selection of the optimum conditions for vacuum drying were based on the desirability function (Pajohi *et al*., 2011).

Stability of bixin during storage: The study of the storage of the finished products (optimum point of the experimental design) was considered at a temperature of 25°C and relative humidity of 70%. The luminosity and UV were varied between 0% and 100% of the total light power using a Memmert ICH260L room

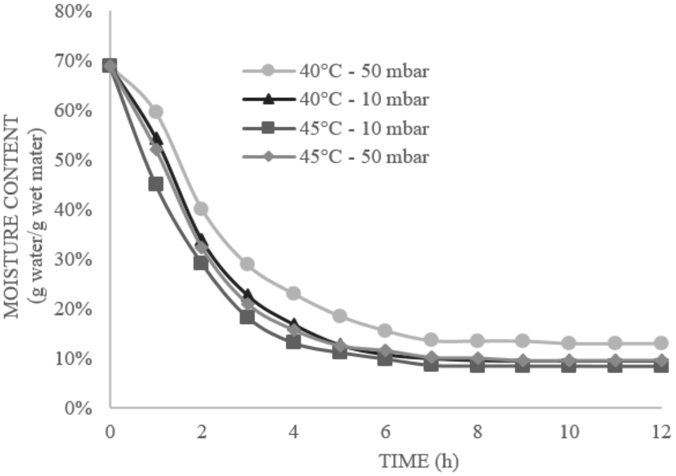


Fig. 1: Drying curves of samples at different temperatures and pressures

conditioning chamber with daylight luminosity control via four fluorescent lamps. The product was monitored for one month, taking samples at 0, 7, 14, 21 and 28 days of storage. The products were placed in four Petri dishes with 5 g of seeds in each one and exposed to the conditions programmed at atmospheric pressure. For each storage time, moisture content and bixin content were estimated according to the methodology described earlier.

RESULTS AND DISCUSSION

Drying kinetics of annatto seeds: The initial MC in fresh annatto seeds was 69.12±0.67% (w.b). The drying curves of annatto seeds at two temperatures and two vacuum pressures are presented in Fig. 1. As expected, the results suggest that decreasing the pressure has positive results on reducing the moisture content and diminishing the processing time. Depending on the drying conditions, the MC varied from 8.42% to 12.98% (w.b) and a reduction of the drying time can be reached by decreasing the pressure at the same temperature. Similar results were found in red currants, where increasing the vacuum pressure presents slower drying kinetics and a higher MC, while decreasing the vacuum pressure increases the water concentration gradient in samples, thus providing faster drying and lower values of the MC (Šumić *et al*., 2016).

Reducing the Moisture Content (MC) is an important criterion that helps to avoid the deterioration of the quality of the sample due to a reduction of the microbial activity that can affect the shelf-life of the product. In general, results suggest that decreasing the pressure has a more remarkable effect than increasing the temperature (Fig. 1). Agreeing with the results of Arevalo-Pinedo and Murr (2007) in carrot and pumpkin, Arevalo-Pinedo and Murr (2006) in pumpkin and Methakhup *et al*. (2005) in gooseberries, the drying vacuum pressure has a great influence on the drying process reflected in the drying kinetics, as it is an important parameter for reducing the moisture content in less time. Additionally, lower pressures present more satisfactory MCs at the same temperature and this can be seen in Fig. 1.

Considering a constant pressure and analyzing the influence of temperature in the drying kinetics, for 10 mbar, the gap between 40°C and 45°C was 1.15%, while for 50 mbar the difference was 3.4%. This suggests that it is important to consider that the temperature has a positive influence accentuating the effect of pressure, thus increasing the drying kinetics and reaching the equilibrium moisture content in less time. Similar results were found by Wu *et al*. (2007) in eggplants and Thorat *et al*. (2012) in ginger, where increasing the drying temperature shortened the drying time to obtain an equilibrium moisture content due to a larger driving force for heat and mass transfer at higher drying temperatures. In Indian gooseberries, Methakhup *et al*. (2005) concluded that higher temperatures provided a difference between the saturated water vapor pressure and the partial pressure of water vapor in air at a given temperature, which is one of the most important forces for drying.

Mathematical modeling: Nonlinear regression analysis was applied to determine the fitting model for every combination of temperature and pressure. The MR and drying time (t) were correlated with mathematical models for vacuum drying. The values of the coefficients and statistical analysis used to select the model (R^2, χ^2 and RMSE) are presented in Table 3, with the best model given in bold letters.

In general, all of the selected thin layer models present a good fit (R^2>0.96), where the RSME was the most important parameter in selecting the model that describes the real drying kinetics due to it representing the standard deviation of the experimental and predicted data for every model. Results show that

Table 3: Fitting statistical results for defined thin layer models in annatto seeds

	Coefficients				Statistical results		
Drying mathematical model	k	a	b	n	R^2	χ^2	RMSE
Experimental conditions: Temperature 40 °C, Pressure 10 mbar							
Lewis	0.391	-	-	-	0.9859	0.000699	0.024938
Henderson and Pabis	0.414	1.037	-	-	0.9763	0.000704	0.023411
Wang and Singh	-	1.888	-0.147	-	0.9875	0.086890	0.259964
Peleg	-	0.906	0.332	-	0.9868	0.001250	0.031185
Page[a]	0.308	-	-	1.482	0.9984	0.000080	0.007916
Experimental conditions: Temperature 40 °C, Pressure 50 mbar							
Lewis	0.296	-	-	-	0.9817	0.000787	0.026461
Henderson and Pabis	0.324	1.044	-	-	0.9844	0.000762	0.024359
Wang and Singh	-	1.655	-0.116	-	0.9876	0.000609	0.021768
Peleg	-	0.860	0.221	-	0.9777	0.001094	0.029172
Page[a]	0.234	-	-	1.508	0.9961	0.000186	0.012055
Experimental conditions: Temperature 45 °C, Pressure 10 mbar							
Lewis	0.528	-	-	-	0.9978	0.000090	0.008979
Henderson and Pabis	0.534	1.009	-	-	0.9979	0.000101	0.008881
Wang and Singh	-	2.040	-0.170	-	0.9685	0.024196	0.137183
Peleg	-	0.970	0.544	-	0.9900	0.000496	0.019646
Page[a]	0.496	-	-	1.154	0.9994	0.000035	0.005226
Experimental conditions: Temperature 45 °C, Pressure 50 mbar							
Lewis	0.321	-	-	-	0.9863	0.000635	0.023766
Henderson and Pabis	0.336	1.022	-	-	0.9870	0.000683	0.023051
Wang and Singh	-	1.782	-0.130	-	0.9954	0.000258	0.014190
Peleg	-	0.927	0.256	-	0.9798	0.001066	0.028798
Page[a]	0.268	-	-	1.365	0.9944	0.000295	0.015156

[a] Selected model.

Page's model exhibited the highest correlation of the R^2 as well as lower values of χ^2 and RMSE for every combination of temperature and pressure. The values of k were from 0.234 to 0.496 and it is important to note that the k value is closely related with the drying rate (Li *et al*., 2017) due to that higher k values present a shorter drying time and faster drying rate in the vacuum drying of annatto seeds. In addition, it can be observed that the k value increased when the pressure decreased due to a lower pressure inside the chamber, thus allowing a lower boiling point of water and enhancing the evaporation of water in the product. The temperature had an impact on the k value at the same pressure, as increasing temperatures also increased the k value. This can be explained through diffusivity, the increase of temperature intensifies the heat and mass transfer driving forces, promoting the dehydration of the sample and similar results were obtained by (Methakhup *et al*., 2005).

The n value varied from 1.154 to 1.508 and exhibited a dependence on the temperature and pressure. As they increased, the n value decreased; this is related to the speed of the exponential model function decreasing and that increasing the drying rate led to reaching the equilibrium MC in less time.

The constants found in this research are in line with the results presented in Yacon (Reis *et al*., 2012), but in previously reported studies on the dehydration of annatto seeds using a convective dryer, the k and n values differed from those found using vacuum drying. The drying kinetics in forced hot air are rather slow and took more time to reach the equilibrium MC, which is reflected in the lower values of Page's constants (Da Costa-Santos *et al*., 2013; Santos *et al*., 2012).

Table 4: Physicochemical properties of fresh annatto seeds

Variable	Mean value
Moisture content (% w.b.)	69±0.01
Aw	0.954±0.003
Bixin content (%)	2.9±0.2
L*	15.77±0.011
a*	20.23±0.029
b*	16.45±0.037

Experimental design: Physicochemical properties of fresh annatto seeds were analyzed to compare the changes in the seeds after vacuum drying. The results are shown in Table 4.

Experimental design shows the interactions between temperature, pressure and time for vacuum drying. Table 5 provides the data generated by the experimental design for every run. These data was analyzed by ANOVA, where variables such as the water activity (Aw), MC and Bixin Content (BC) were statistically significant ($p<0.05$). The total color change and bulk density were not statistically significant ($p>0.05$). Table 6 shows the quadratic models for every response variable (statistically significant) according to the experimental design, where β_0 represents the intercept coefficient, β_1 represents the temperature, β_2 represents the pressure, β_3 represents the time, β_{12} represents the interaction between temperature and pressure, β_{13} represents the interaction between temperature and time, β_{23} represents the interaction between pressure and time and β_{11}, β_{22} and β_{33}, represent the quadratic terms of temperature, pressure and time, respectively.

Table 5: Experimental design and data for response surface analysis

	Independent Variable					Response Variable		
Run	Temperature [°C]	Pressure [mbar]	Time [h]	Bulk density [g/L]	Aw	MC [%w.b.]	Bixin content [%]	ΔE
1	42.5	10	6	0.355±0.011	0.500±0.008	9.6±0.2	8.7±0.03	2.86
2	45	10	9	0.420±0.003	0.397±0.004	9.5±0.1	8.6±0.05	1.34
3	45	50	9	0.351±0.008	0.504±0.002	13.3±0.4	7.8±0.01	1.24
4	42.5	30	9	0.346±0.013	0.430±0.003	10.9±0.2	7.8±0.03	1.35
5	40	30	12	0.314±0.005	0.522±0.003	8.6±0.1	8.1±0.13	1.57
6	42.5	10	12	0.303±0.016	0.285±0.006	7.9±0.2	10.1±0.03	1.08
7	42.5	30	9	0.338±0.019	0.438±0.006	11.5±0.5	7.1±0.05	0.90
8	42.5	50	6	0.365±0.012	0.761±0.002	18±0.6	6.2±0.07	2.97
9	45	30	12	0.391±0.014	0.438±0.001	8±0.3	8.2±0.04	2.14
10	40	30	6	0.350±0.021	0.619±0.008	12.1±0.4	7.9±0.01	1.25
11	40	50	9	0.422±0.007	0.568±0.004	11.3±0.2	7.6±0.04	1.31
12	42.5	30	9	0.439±0.023	0.435±0.010	10.3±0.1	8.4±0.03	1.23
13	42.5	50	12	0.375±0.013	0.535±0.016	10.1±0.3	7.3±0.01	2.04
14	40	10	9	0.320±0.005	0.390±0.008	10.8±0.3	8.1±0.07	1.12
15	45	30	6	0.335±0.015	0.506±0.002	11.6±0.2	8±0.03	2.12

Table 6: Estimated coefficients of the second order polynomial model for all response variables

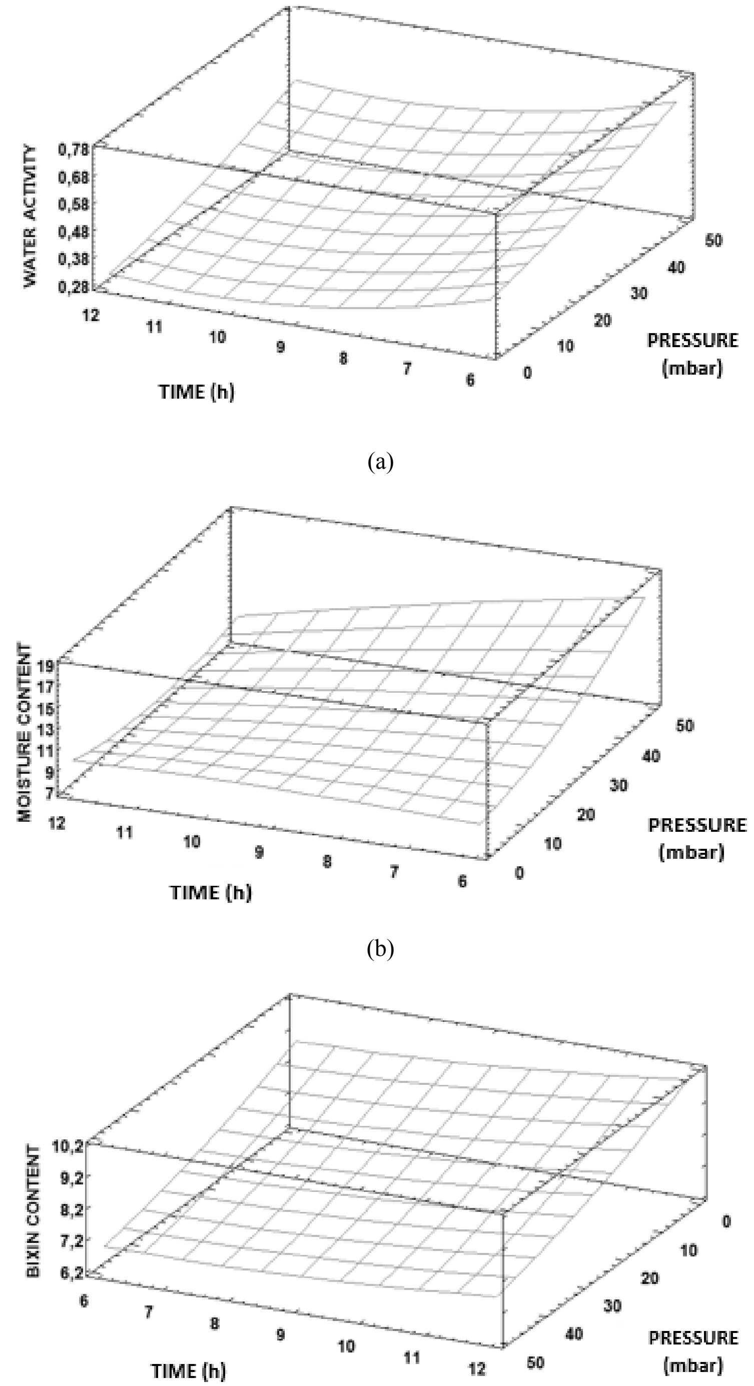
	Independent variable		
Regression coefficient	Water activity	Moisture content (%)	Bixin content (%)
β_0	6.15931	-117.563	39.2896
Linear			
β_1	-0.223617	6.315	-1.49667
β_2	0.0181037^a	-0.499375^a	0.0125^a
β_3	-0.207808^a	0.870833^a	-0.175
Cross product			
β_{12}	-0.000351	0.0165	-0.0015
β_{13}	0.0009666	-0.00333333	0
β_{23}	-0.000045	-0.0258333^a	-0.00125
Quadratic			
β_{11}	0.00250267	-0.08	0.0186667
β_{22}	0.0000364792	0.0020625	0.000354167
β_{33}	0.00793796	-0.0361111	0.0185185
R2 (%)	89.34	92.01	79.6

a Significant at 0.05 level

Effects of independent variables on the drying process: The response surface is shown in Fig. 2 for the following statistically significant response variables: water activity, moisture content and bixin content. The bulk density is closely related with the MC in the seed drying process. Previous reports on mahogany seeds and kernels (Aviara *et al.*, 2014) have related the decrease of the bulk density with reducing the MC. Due to the drying time interval in the experimental design (6-12 h), it can be seen in Fig. 1 that the MC did not change too much between 6 and 12 h. Therefore, the bulk density would not change enough to present a statistically significant effect. Similar results were found for the total color change, as prolonged high temperature caused degradation of the pigments (Šumić *et al.*, 2016) and higher total color changes due to a browning effect (Reis, 2014). The color in annatto could also be affected by light and oxygen (Gallardo-Cabrera and Rojas-Barahona, 2015), but high temperatures were not used in this experiment. Furthermore, the seeds were dried in a dark chamber with low pressures, which means a low oxygen content inside, thus explaining why the color was not statistically significantly affected.

Water activity: Figure 2a illustrates the water activity according to the drying conditions and Table 6 shows that pressure and time were statistically significant variables for the Aw ($p<0.05$). This parameter is closely associated with moisture content, as is the ratio of the partial pressure of water vapor in the product to the partial pressure of pure water (Michalska *et al.*, 2017). This parameter is useful to predict the stability of the product during shelf-life, as microorganisms usually grow between 0.99-0.98, while microbes stop growth at Aw>0.9. Meanwhile, some fungi cannot reproduce when Aw is below 0.62 (Šumić *et al.*, 2016). Lower pressure values promote the evaporation of water on the sample, increasing the solute concentration in seeds and decreasing the amount of water available to grow microorganisms. A prolonged processing time helps the drying process by increasing the period to evaporate more water, implying better values that enhance the shelf-life of the product (Methakhup *et al.*, 2005). For dry annatto seeds, it was found that Aw was between 0.761±0.002 and 0.285±0.006, which is favorable for the product during storage. Similar results were found in sour cherries, where lower pressures and higher temperatures during a prolonged drying time reach lower Aw values (Šumić *et al.*, 2013).

Moisture content: Pressure and time, as well as their interaction, were statistically significant ($p<0.05$). Figure 2b shows the surface response for this variable. The MC is a quality parameter due to that the amount of available of water promotes the proliferation of microorganisms and spoilage of food. A stable product has a MC of approximately 14%, whereas greater values can present a shorter shelf-life (Durance, 2002). Pressure decreases the boiling point of water in the sample, causing effective evaporation in the product and thus more water evaporates during prolonged



(a)

(b)

(c)

Fig. 2: Response surfaces for significant parameters; a): water activity; b): moisture content (%, w.b); and c): bixin content (%)

drying processes. The experimental procedure shows a significant reduction in the original MC (69%±0.01% w.b.) and the lowest value of the experimental design (7.9%±0.2% w.b.), thus demonstrating an effective method of drying through vacuum drying. Reported studies on red currants agreed with this research that pressure and time are significant parameters, exhibiting important reductions in MC values when the pressure decreased and the drying time increased in the drying parameters (Šumić *et al*., 2016).

The effect of the interaction between time and pressure on the MC was statistically significant ($p<0.05$). Decreasing the pressure and increasing the processing time reduced the MC by increasing the

Table 7: Comparison between the optimum and experimental drying

Response variable	Optimum	Experimental	Lyophilized
Water activity	0.358	0.282±0.07	0.2935±0.03
Moisture content (% w.b.)	7.588	7.65±0.16	7.92±0.25
Bixin content (%)	9.613	8.70±0.08	7.05±0.02

water evaporation rate due to the reduction of the boiling point. A prolonged time allows the phenomenon takes place in the sample during a longer period to evaporate more water and reduce the MC and this result agreed with those found for eggplants (Wu *et al*., 2007) and gooseberries (Methakhup *et al*., 2005).

Bixin Content (BC): Pressure is the principal parameter that significantly affects the bixin content during the vacuum drying process (Fig. 2c). Bixin is the most important component of annatto seeds and the pressure helps the water to move out of the sample, thus increasing the kinetics in water molecules and finally promoting evaporation. This phenomenon helps this oleoresin to concentrate and expel water impurities that could be reflected in bixin analysis. The bixin content changes greatly between fresh (2.9%±0.17) and the highest experimental value obtained (10.1%±0.03%), demonstrating that lower pressure values have a great influence on the enhancement of the bixin concentration. This can be attributed to the nature of bixin ($C_{25}H_{30}O_4$), with 11 double bonds in its structure. Thus, when this carotenoid is exposed to free radicals or oxidizing agents, the electron transfer in the presence of double bonds or the hydrogen abstraction from the bixin molecule cause the breaking of the chain of the antioxidant (Chisté *et al*., 2011). Due to the low pressures used in the experiment, the presence of oxygen was low in the drying chamber, thus avoiding bixin degradation. On the other hand, this is the first time reporting the effect of the pressure of vacuum drying on the BC and therefore, there are not any previous reports to compare with the results found in this research.

Optimization: An optimization of multiple responses was carried out using Derringer's desire function methodology on response variables. The statistically significant variables (Aw, MC, BC) were used to feed the software and the impact factors used were 4, 4 and 5, respectively. The main goal for every statistically significant response variable was to minimize the Aw and MC as well as maximize the BC. The optimum conditions for annatto seeds were 45°C, 10.453 mbar and 12 h. Table 7 shows the predicted values for the optimum conditions in annatto seeds and the values contrasted with experimental procedures and lyophilization, a type of drying process that can be compared to vacuum drying. Results showed a small difference between predicted and experimental values, which means a successful optimization of the experiment. Although lyophilization presents good results, compared with vacuum drying, the values were slightly under the found values, validating the use of vacuum drying as an adequate technology for annatto seeds. Nevertheless, optimization on annatto seeds using lyophilization has never been performed and the parameters used were standard for other food matrices. Lower bixin content values using lyophilization can be explained through the heating rate during sublimation, for which overheating the product could make sublimation phenomena faster but also too high of a temperature rate can collapse the pore structure and deteriorate the quality properties of the product, which is related to the glass transition temperature (Lopez-Quiroga *et al*., 2012).

Stability of bixin during storage: Annatto seeds dried under the optimum drying conditions were analyzed under storage. Decreases in the BC throughout the storage time can be observed, being more marked in the seeds exposed to direct light and oxygen. For both conditions, it could be seen that since the first moment dried seeds are stored, they begin to degrade the compound of interest, possibly because the carotenoids are by nature are unstable compounds and bixin is one of them (Chisté *et al*., 2011).

Degradation by oxygen occurs through electron transfer, forming a radical and then, this radical reacts with oxygen, forming an oxidation product (Dwivedi and Pillai, 2014). It had been reported that direct illumination has a strong destructive effect on bixin more than oxygen (Najar *et al*., 1988) and it is also well known that 9'-cis-bixin is an effective quencher of singlet oxygen and a sensitizer, promoting autoxidation and photosensitized oxidation. Photosensitized reactions involve the absorption of photons by a sensitizer molecule, resulting in an energy rich state, producing the alteration of another molecule. In this triplet state, the carotenoid returns to the ground state with the liberation of heat (Montenegro *et al*., 2004). Nevertheless, fast reversible isomerizations and no reversible degradation can occur for the rapid transition of the molecule to the equilibrium mixture of forms (Scotter *et al*., 2001). Additionally, it is common to find bleaching and the formation of various oxidation products after a prolonged time of exposure to photosensitization (Montenegro *et al*., 2002). This explains the loss of bixin pigment due to the chemical process because of the energy transferred to the carotenoid by physical quenching, which promotes dissipation by rotational and vibration interactions, thus generating oxidation products (Rios *et al*., 2009).

Figure 3, the equations describing the loss of bixin in light and dark conditions are shown. Both equations present a coefficient of adjustment up to 0.95, which

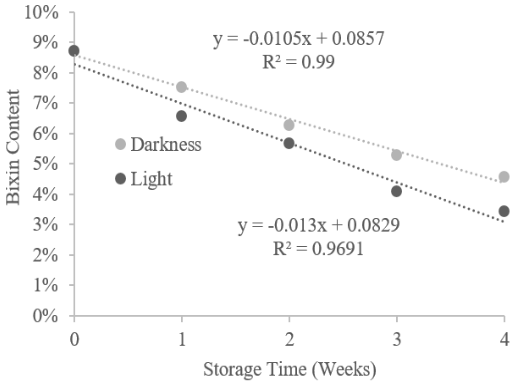


Fig. 3: Bixin content during storage conditions

suggests that the equations can predict the loss of the carotenoid at any time of storage under the conditions evaluated.

Although a decreasing trend occurs in both conditions, the slope is higher (-0.013) under light conditions with respect to dark conditions (-0.0105). It is inferred that by modifying the luminosity conditions, the rate of loss of bixin can be increased, as occurs in the illuminated chamber, or decreased in the case of the dark chamber, whereby it is concluded that the best conditions to preserve the carotenoid bixin during storage are under total darkness. Other investigations report light sensitiveness in microencapsulated bixin, where notorious bixin stability of two orders of magnitude is reported under darkness conditions compared to under light conditions (Barbosa *et al*., 2005). The storage stability was measured in nano-encapsulated bixin under ambient conditions, where the samples were kept in amber glasses. High variation in the bixin content occurs, especially during the first days of storage, due to the presence of oxygen in the bottles and unprotected bixin in the continuous phase. After the 7th to 28th days of storage, there was no significant variation in the BC (Lobato *et al*., 2013).

CONCLUSION

The results suggest that using vacuum drying on annatto seeds has an effect on the drying kinetics, dismissal time of the process and reaching a suitable moisture content and water activity that allows a stable product during the shelf-life. Additionally, for the interaction between temperature and pressure, achieving a lower moisture content in a shorter time cannot be obtained using conventional drying methods as the pressure is the most important parameter that influences the drying process and bixin content. Storage under light and darkness conditions presented a decreasing bixin content in annatto seeds. Moreover, the loss was less than under light conditions.

ACKNOWLEDGMENT

The authors thank the Universidad Nacional de Colombia and all the laboratory technicians team, who took part in the guide and development of this research.

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