

Full Length Research Paper

Influence of the storage conditions on moisture and bixin levels in the seeds of *Bixa orellana* L.

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This study was conducted to analyze the changes in the moisture and bixin levels in the seeds of *Bixa orellana* L. under four storage conditions: Storage without tarp (SB), storage over tarp (BD), seeds fully covered with tarp (BE) and storage in a room chilled to 4°C (OB). For 13 weeks, moisture content and bixin levels were monitored. The decreases in bixin after 13 weeks of storage at the different storage modes SB, BD, BE and OB were respectively 28.6, 25.9, 15.6 and 11.0%, while the increments in the moisture content were respectively 63.4, 57.0, 55.7 and 31.3%. No matter the storage mode, the fall of bixin was proportional to the moisture absorption. The storage mode OB brought the smallest loss of bixin and the smallest increment of moisture content, followed by BE. In tropical areas, however, the full covering of seeds with tarp would be more effective both in terms of cost and practicality. Moreover, the establishment of sorption isotherms allowed to suggest an optimal storage of the seeds of *Bixa orellana* L. in a dry enough environment having a relative humidity comprises between 12 and 30%.

Key words: *Bixa orellana* L., bixin levels, storage conditions, moisture, water activity.

INTRODUCTION

Côte d'Ivoire is located in West Africa, in the area between the tropics, at the north of the Gulf of Guinea. Its main export crops are annuity products such as coffee, cocoa, cotton and palm oil. However, the drastic fall in the prices of these raw materials from the nineties has gradually led the Ivorian farmers to shift to new crops, including the *Bixa orellana* L. as indicated in reports by the Ministry of Agriculture and Animal Resources of Côte d'Ivoire and OCDE (MARA, 1999; OCDE, 2002). *B. orellana* L. is mainly grown for its seeds which are

covered with a dye composed of 70 to 80% of bixin and 20 to 30% of orelline (Dora and Flores, 1988; Aparnathi et al., 1990). Its chemical composition characterized by the presence of carotenoids (bixin and norabixine), flavonoids and terpenoids (Satyanarayana et al., 2003; Jondiko and Pattenden, 1989) accounts for its therapeutic and food interests (Chengaiyah et al., 2010; Antunes et al., 2005; Agner et al., 2005; Fleischera et al., 2003).

Moisture absorption and mold growth remain the major

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Table 1. Volume of distilled water added to construct the absorption isotherm.

Receptable number	Water volume added to the seeds (ml)
1	0.0
2	0.3
3	0.6
4	0.9
5	1.2
6	1.5
7	1.8
8	2.1
9	2.4
10	2.7

concerns that producers have to contend (Mara, 1999). The deterioration of seeds during drying and, especially, during storage prior export results in poor marketability products (Lavelli et al., 2007). Needless to say, post-harvest storage conditions of seeds are critical parameters in the conservation of the physicochemical properties especially as regards the content of natural dyes (Fleischera et al., 2003).

Thus, a pilot study was conducted to study the evolution of the amount of dye depending on the storage conditions.

MATERIALS AND METHODS

Sampling

The biological material consisted of annatto seeds (*B. orellana* L.) from Bondoukou and Tanda, two regions of high production, in the Northeast of Côte d'Ivoire. These seeds were collected during the high season and the early season (January-October) of the year 2009. For each storage mode, three batches of 12 bags of 50 kg each were made and four storage modes were considered: storage without tarp (SB), storage over tarp (BD), seeds fully covered with tarp (BE) and storage in a room chilled to 4°C (OB). The different batches were stored for 13 weeks. Before storage, samples were taken from batches at the rate of one sample (1 kg) per week. Samples were sent to the laboratory for determination of moisture, pigment (bixin) and water activity. Each sample was analyzed in triplicate and a total of 1008 trials were analyzed.

Determination of moisture

The moisture content was determined according to the method described by AOAC (1990). A sample of 5 g of annatto seed (*B. orellana* L.) was introduced into an aluminum box and then placed in an oven at 105°C till a constant weight was reached. Each test was performed in triplicate. The result was expressed as follows:

$$\text{Moisture (\%)} = 100 - [(M1 - M0) \times 100 / Me]$$

M0 is mass of the empty box; M1 is mass of the box containing the sample after drying and cooling and Me is sample mass.

Extraction of pigments from the seeds of *B. orellana* L.

A volume of 50 ml of KOH 5N was then placed in a stirring system from Lab-Line Instruments which alternates phases of agitation and rest for 24 h. The flask was finally adjusted to the mark with distilled water. The resulting solution was used for colorimetric analysis (McCormick, 1996).

Determination of bixin levels

The determination of bixin was performed using the method of McCormick (1996). A colorimeter from Milton Roy Spectronic was calibrated with a solution of 0.075N KOH and an aliquot of 3 ml was introduced into a flask of 100 ml and adjust to the mark with distilled water. Three absorbance measurements were made at a wavelength of 454 nm. The percentage of bixin was calculated using the following formula:

$$\text{Bixin\%} = (AxV1xV2)/(MxExV3)$$

A is absorbance; M is mass of sample; E is extinction coefficient of bixin = 3200; V1 is initial volume of dilution (ml); V2 is final volume dilution (ml), and V3 is volume of aliquot (ml).

Construction of sorption isotherms

The relationship between water content and water activity of a product at a constant temperature can be represented by the sorption isotherm that allows expressing the phenomenon of hysteresis. A hygrometer from HygroLabRotronic was used to measure water activity according to the method by McCormick (1995).

Absorption isotherm

A sample of 5 g seed of *B. orellana* L. was placed in 10 Aw containers void of any trace of water. Increasing volumes of distilled water were added to each container (Table 1). After two minutes of soaking, measurements of water activity and moisture were carried out. A plot of the evolution of water activity (Aw) as a function of moisture content was constructed.

Desorption isotherm

A volume of 500 ml of distilled water were added to 100 g of seeds of *B. orellana*. After two minutes of soaking, water was removed through a sieve. Then, 5 g of wet seeds were then placed in a series of 10 aluminum crucibles and heated in an oven at 105°C for two hours. After this drying, the crucibles were successively removed from the oven at regular intervals of 10 min. The moisture and water activity were then determined for each pot of the series and a curve of changes in water activity as a function of moisture content was constructed.

Statistical analysis

Data obtained were seized under Excel software then statistically analyzed using Statistica version 7.1, analysis of variance (ANOVA). Averages and standard deviations of the analyzed parameters were classified using Newman-Keuls test and mean values were compared used student test. Differences were considered to be statistically significant when $P < 0.05$ level.

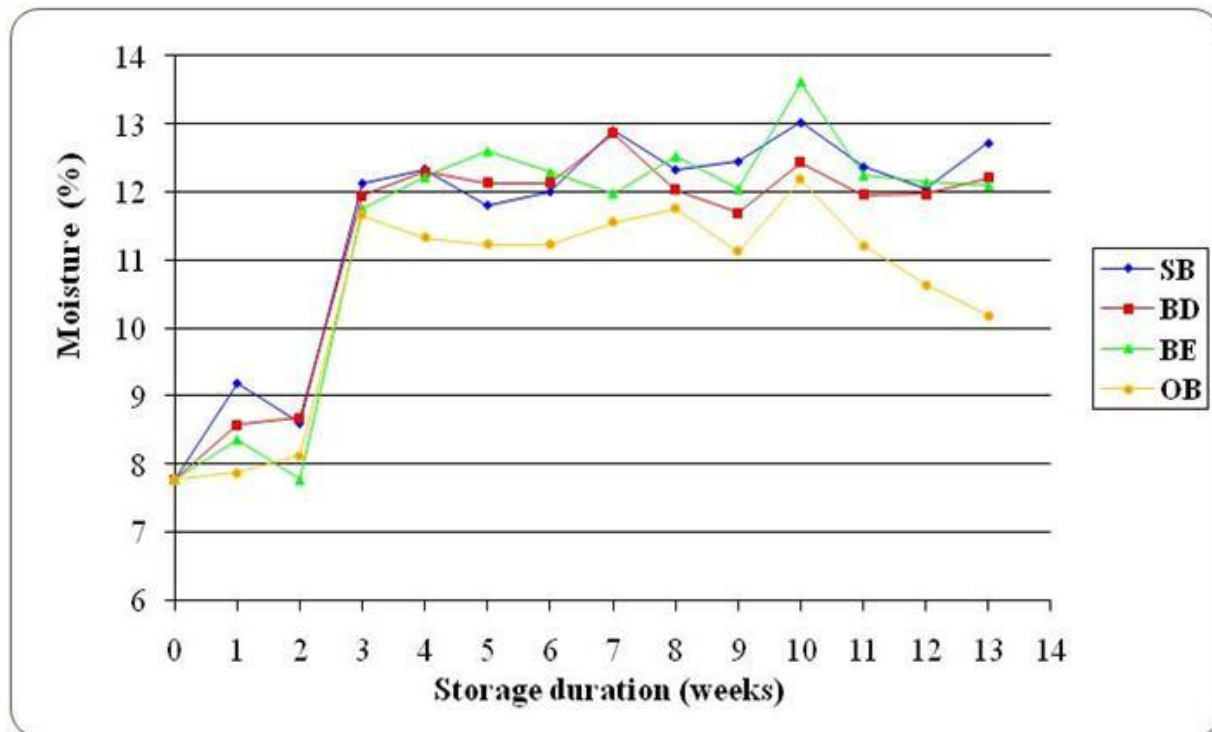


Figure 1. Evolution of moisture content depending on the storage duration (storage without tarp (SB), storage over tarp (BD), seeds fully covered with tarp (BE) and storage in a room chilled to 4°C (OB).

RESULTS

Evolution of moisture and bixin contents during storage

The results of changes in moisture percentage as a function of storage duration are shown in Figure 1. Figure 2 shows the changes in bixin levels in the same conditions. This study shows a decrease in bixin levels and an increase in moisture, whatever the storage method (Figures 1 and 2). The initial rates of bixin and moisture before storage were respectively 2.42, 2.43, 2.66 and 2.79% for the storage modes SB, BD, BE and OB that was a respective loss of bixin of 28.6, 25.9, 15.6 and 11.0%. The final moisture contents were respectively 11.5, 11.3, 11.3 and 10.5% for the storage modes SB, BD, BE and OB that was a respective moisture absorption of 63.4, 57.0, 55.7 and 31.3%. Considering the four storage modes, the significance of the reduction of bixin was as follow: $OB < BE < BD < SB$. Generally, this decrease is made while humidity increases (Figures 1 and 2). The storage mode that causes the smallest loss of bixin and the smallest increase in moisture content was the storage in room chilled to 4°C (OB), followed by the storage of seeds fully covered with tarp (BE). The storage without tarp is responsible for the most important loss of pigment and the highest increase in moisture. The test of the least significant difference revealed a significant difference at 5% between the averages of loss of bixin for the different storage modes.

Sorption isotherm curves

Figure 3 shows the results of absorption isotherm of *B. orellana* seeds. Water activity and moisture absorption increase in same time during *B. orellana* absorption. This absorption followed hyperbola form. The first was an ascending phase of weak slope with moisture absorption from 5 to 11%, corresponds to a water activity of 0.119 to 0.95; and a second vertical phase from 11 to 40% of moisture absorption corresponding a water activity of 0.97. Desorption isotherm of *B. orellana* seed describes a linear regression and curve present three distinct phases (Figure 4), the first ascending phase with moisture absorption from 10 to 31% corresponds to a water activity between 0.119 and 0.319; the second phase of weak slope shows the water activity from 0.319 to 0.9 and correspond to moisture absorption between 31 to 35%; and the last phase with water activity between 0.9 to 0.97 corresponding a moisture absorption from 35 to 80%.

DISCUSSION

Regarding humidity, the storage mode OB differed significantly from the other storage modes BE, BD and SB. This study clearly shows the influence of seeds moisture on the reduction of bixin. Gloria et al. (1995) showed deterioration in the stability of bixin in the presence or absence of air and light. They note that the half-life of bixin was less than 8 days when storage

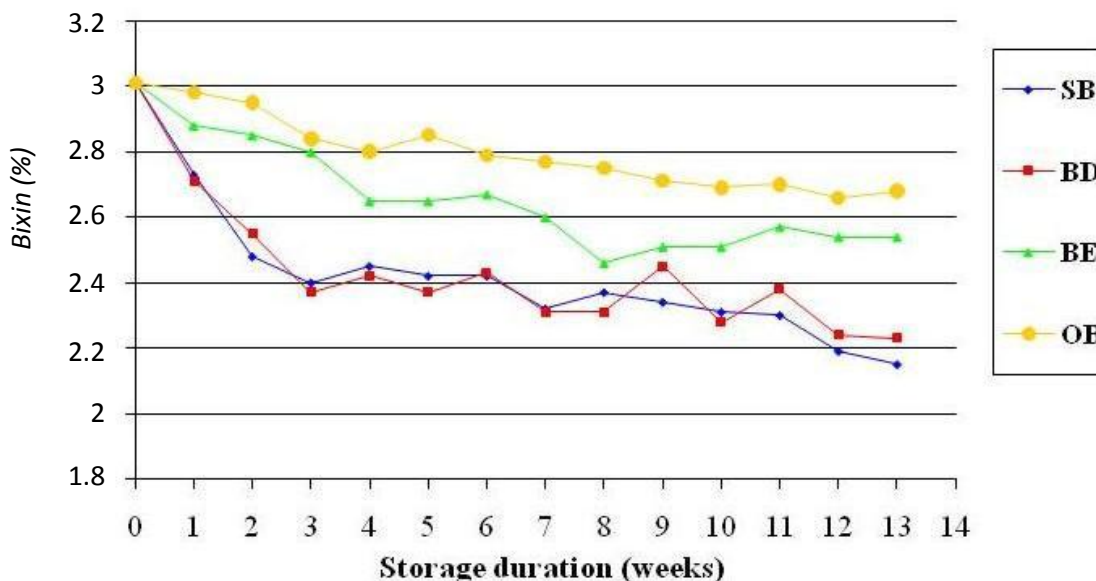


Figure 2. Evolution of bixin rates depending on the duration of storage (storage without tarp (SB), storage over tarp (BD), seeds fully covered with tarp (BE) and storage in a room chilled to 4°C (OB).

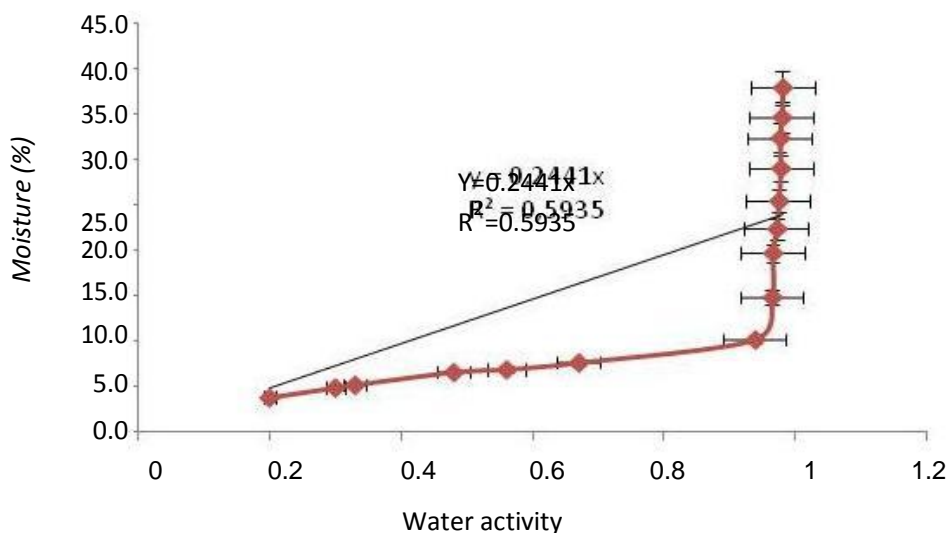


Figure 3. Absorption isotherm of *Bixa orellana* L. seeds.

occurs in the presence of light and air, while it was over 55 days in the absence of light and under nitrogen. Several authors have also demonstrated a degradation of bixin and β -carotene of dehydrated carrots during storage (Goldman et al., 1983; Najar et al., 1988; Ribiero et al., 2005; Lavelliet al., 2007). The loss of bixin, activated by light, is caused by its oxidation in the presence of air (Di Mascio et al., 1989; Bradley and Min, 1992; Gloria et al., 1995).

The huge concern of degradation of *B. orellana* and mostly the lack of technical management and definition of

quality standards according to permissible humidity of seeds have led producers to abandon its cultivation. Thus, it is of utmost importance for the Ivorian authorities to become more involved in the sector by organizing, training and educating all the stakeholders. Absorption and desorption isotherms were different because of the irreversible phenomena of porosity. The linear regression curves associated with them can predict the value of water activity for seed moistures known. Thus, at the beginning of the storage, the seeds had moisture content of 7.78% and depending on whether it is based on the

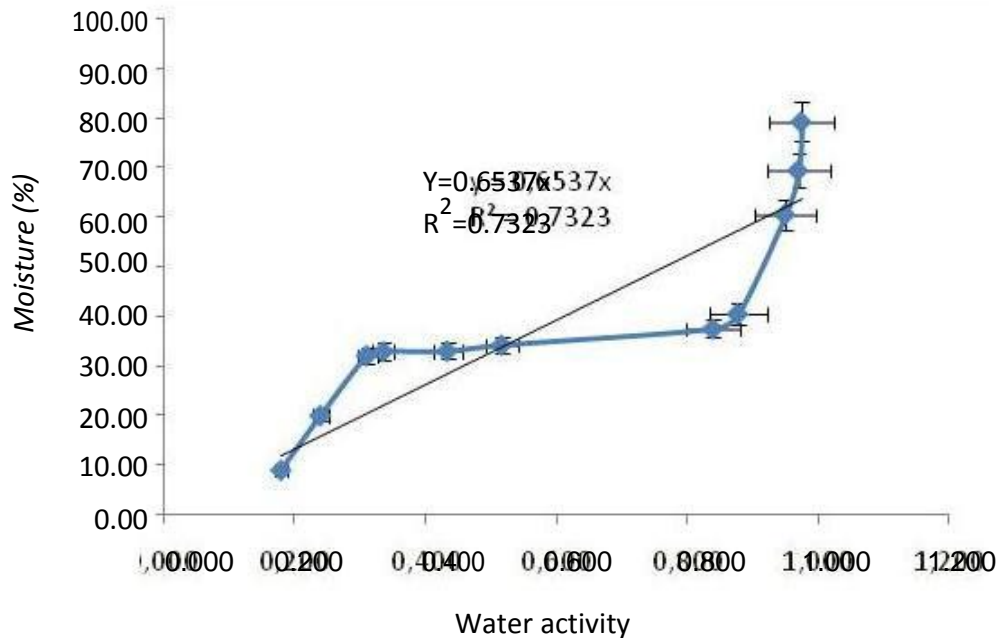


Figure 4. Desorption isotherm of *Bixa orellana* L. seeds.

absorption isotherm or desorption, moisture corresponded to a water activity between 0.119 and 0.319. To avoid moisture absorption, seeds should be stored in an environment where the moisture is in equilibrium with their water activity. We note therefore, the corresponding relative moisture was between 11.99 and 31.9%.

Conclusion

This study showed a close relationship between changes in bixin and moisture contents of seeds of *B. orellana*. The storage in a room chilled to 4°C appeared to be the most appropriate type of storage for these seeds, but too expensive for Ivorian farmers. They could use the second most appropriate mode storage instead: the storage of seeds fully covered with tarp. Finally, the construction of sorption isotherms allowed to suggest a storage of seeds in a dry environment with humidity between 12 and 30%.

REFERENCES

- Agner AR, Bazo AP, Ribeiro LR, Salvadori DM (2005). DNA damage and aberrant crypt foci as putative biomarkers to evaluate the chemopreventive effect of annatto (*Bixaorellana* L.) in rat colon carcinogenesis. *Mutat. Res.* 582:146-154.
- Antunes LM, Pascoal LM, Bianchi M, Dias FL (2005). Evaluation of the clastogenicity and anticlastogenicity of the carotenoid bixin in human lymphocyte cultures. *Mutat. Res.* 585:113-119.
- AOAC (1990). Official methods of analysis. Association of Official Analytical Chemists 13th edition, Arlington, Virginia, USA.
- Aparnathi KD, Lata R, Sharma RS (1990). Annatto (*Bixa orellana* L.) its cultivation, preparation and usage. *Int. J. Trop. Agric.* 8:80-88.
- Bradley DG, Min DB (1992). Singlet oxygen oxidation of foods. *Crit. Rev. Food Sci. Nutr.* 31:211-236.
- Chengaiyah B, Rao KM, Kumar KM, Alagusundaram M, Chetty CM (2010). Medicinal importance of natural dyes a review. *Int. J. Pharm. Tech. Res.* 2:144-154.
- Di Mascio P, Kaiser S, Sies HE (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* 274:532-538.
- Dora R, Flores EM (1988). Le beurre, la margarine, l'huile, le riz, le bonbon et certains fromages à teinte rouge contiennent le pigment de rocou. *Species Plantarum* 1:422-575.
- Fleischera TC, Ameadea EPK, Mensaha MLK, Sawerb IK (2003). Antimicrobial activity of the leaves and seeds of *Bixaorellana*. *Fitoterapia* 74:136-138.
- Gloria MBA, Vale SR, Bobbio PA (1995). Effect of water activity on the stability of bixin in an annatto extract-microcrystalline cellulose model system. *Food Chem.* 52:389-391.
- Goldman M, Horev B, Saguy I (1983). Decolorization of beta-carotene in model systems simulating dehydrated foods. Mechanism and kinetic principle. *J. Food Sci.* 48:751-757.
- Jondiko IJO, Pattenden G (1989). Terpenoids and an apocarotenoid from seeds of *Bixaorellana*. *Phytochemistry* 28:3159-3162.
- Lavelli V, Zanoni B, Zaniboni A (2007). Effect of water activity on carotenoid degradation in dehydrated carrots. *Food Chem.* 104:1705-1711.
- MARA (1999). L'agriculture ivoirienne à l'aube du 21^{ème} siècle. Rapport interministériels des Ministères de l'Agriculture et des Ressources Animales, de l'Environnement et de la forêt, de l'enseignement Supérieur et de la Recherche scientifique. Abidjan, Côte d'Ivoire. P. 243.
- McCormick (1995). Determination of water activity. McCormick and Company, Inc. Manual of technical methods and procedures. Baltimore, USA.
- McCormick (1996). Extractable color in Annatto *Bixaorellana*, oil soluble and encapsulated Annatto samples. McCormick and Company, Inc. Manual of technical methods and procedures. Baltimore, USA.
- Najar SV, Bobbio FO, Bobbio PA (1988). Effects of light, air antioxidants and pro-oxidants on annatto extracts (*Bixa orellana*). *Food Chem.*

- 29:283-289.
- OCDE (2002). L'économie locale de Bondoukou: comptes, acteurs et dynamisme de l'économie locale. Rapport de l'Organisation de Coopération et de Développement Economique OCDE SAH/D521/2001, Paris, France. P. 104.
- Ribiero JA, Oliveira DT, Passos ML, Barrozo MAS (2005). The use of nonlinearity to discriminate the equilibrium moisture equations for *BixaOrellana* seeds. J. Food Eng. 66:63-68.
- Satyanarayana A, Rao PGP, Rao DG (2003). Chemistry, processing and toxicology of annatto (*Bixa orellana* L.). J. Food Sci. Tech. Mys. 40:131-141.