Bromatological analysis of annatto (*Bixa orellana* L.) seeds

Avalos-Fernández J. M.1; Del Rivero-Bautista N.2*; Hernández-Nataren E.2; Acosta-Pech R. G.2; Pereyra-Susan J. P2

[1] Instituto Tecnológico Superior de Comalcalco. Carretera vecinal, Paraíso-Comalcalco Km 2, Ra. Occidente 3ra sección, Comalcalco, Tabasco, CP 86650.
[2] Colegio de Postgraduados Campus Tabasco. Periférico Carlos A. Molina S/N Km. 3, Periférico Carlos A Molina SN, Ranchería Río Seco y Montaña, Heroica Cárdenas, Tabasco, CP 86500.
* Correspondence: rnidya@colpos.mx

ABSTRACT

Objective: to carry out a bromatological and physicochemical analysis of local annatto seeds and a commercial paste.

Materials and Methods: local annatto seeds to which a physicochemical analysis was carried out where ash, humidity (weight difference), dry matter, proteins were determined. ADF and NDF, fat, and in addition, bromatological analysis was carried out on the samples and the commercial pasta.

Results: sample M3 (dark heart-shaped annatto without filaments) presented the highest values. The bixin content was recorded with 4.09% in sample M2 (heart-shaped red annatto without filaments) and the commercial paste was the lowest with 0.56%.

Limitations and Implications of the study: the importance of performing the bromatological and physical-chemical analysis of annatto seeds of local genotypes determined which of the local samples and the commercial paste are the ones that contain the greatest amount of bixin.

Findings and Conclusions: Sample M3 (dark heart-shaped annatto without filaments) presented the highest values. The highest bixin content was found in the smooth heart-red variety and the lowest value in the commercial pasta.

Keywords: Accessions, foods, proximal analysis, bixin.

Citation: Avalos-Fernández, J. M., Del Rivero-Bautista, N., Hernández-Nataren E., Acosta-Pech, R. G. & Pereyra-Susan J. P. (2023). Bromatological analysis of annatto (*Bixa orellana* L.) seeds. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i12.2557

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: March 24, 2023.
Accepted: October 28, 2023.
Published on-line: December 27, 2023.

Agro Productividad, 16(12). December. 2023. pp: 65-72.

INTRODUCTION

Annatto (*Bixa orellana* L. (Bixaceae) is a perennial shrub whose seeds accumulate a high content of the apocarotenoid pigment bixin, a dye used for its pigmenting qualities, from pre-Columbian times to date. Likewise, bixin is economically and culturally important, since it is consumed in large quantities in Mexico and in the world. The commercialization of this pigment is mainly intended for use in the food, pharmaceutical, textile and cosmetic industries (Rivera-Madrid, 2021). Color, in food, plays an important role from the appearance point of view, which is why colorants as food additives are relevant. They are often used to highlight the natural color of food and others to restore the color lost during handling for preservation. he latter is the case, for example, with strawberry and pea preserves, which would be unattractive and unappetizing without

dyes. Food coloring was already practiced in Roman and Egyptian times (La química y la alimentación, 2018).

Natural dyes are generated by microorganisms, plants, animals, or minerals; economically they represent 940 million dollars in sales in the world market of dyes per year, and due to the consumer's caution for the consumption of products that alter or damage their health, it grows around 4% per year. Natural pigments are those obtained from sources present in nature, used to provide color in some products (Rivera-Madrid, 2021). They are subject to the same quality and toxicological safety testing as synthetics, but the FDA and other government agencies do not require them to be certified for chemical purity, and therefore refer to them as non-certified color additives (Camacaro *et al.*, 2018). In Mexico, there are standards for the food industry that regulate the use of bixin in foods (cheeses, yogurts, meats, creams, margarines, etc.); which establish the permitted doses or concentrations depending on the food and mention that this colorant does not contain toxic substances that may cause illness to the consumer and that it is of vegetable origin. These Mexican Standards are NOM-086-SSA1-1994, NOM-120-SSA1-1994, NOM-131-SSA1-1995, NOM-147-SSA1-1996, NOM-185-SSA1-2002, NOM-213-SSA1-2002 (FDA, 2001; Sahaza, 2001). The chemical and biological analysis of food began its operation as a science in the 19th and 20th centuries, with the purpose of making known the characteristics and nutritional value of food (Acero, 2007). The information obtained through bromatology is critical for the assimilation of the factors that condition the properties of foods and, in the same way, for food processing to be safe, nutritious, and pleasant for the consumer; since then, the improvement of the quality, quantity and availability of food supply worldwide has been introduced (Acero, 2007). Its importance lies in the economic, hygienic and legislative aspects, which is why it is not enough on its own, since it is essential to complement its execution with other disciplines, taking into account the assessment of the nutritional properties and composition of natural and processed foods and their possible adulterations; chemical analysis of the quantitative content of lipids, carbohydrates, vitamins, proteins and minerals present in the different foods; also, the technical regulation of the sanitary sale of food; as well as industrial production, seriation and transport.

Likewise, investigate the causes that induce and accelerate food alterations and through this develop preventive measures to avoid food from being a vehicle for microorganisms, toxins or any substance harmful to health (Salazar, 2014).

This research contributes to the knowledge of local annatto samples that have potential bixin content in their seeds and can be cultivated. The specific objective of this work was to carry out the bromatological and physicochemical characterization of annatto seeds from two communities in the municipality of Cunduacán, Tabasco and a commercial paste.

MATERIALS AND METHODS

Annatto seed samples (Figure 1) collected in two communities in the municipality of Cunduacán, Tabasco, were used as plant material: six corresponded to the Yoloxochitl 3rd section community and two samples to Monte Grande. The samples were placed in paper bags with a capacity of 500 g and labeled; each sample weighed approximately 200 g.

Figure 1. Annatto samples and seeds (*Bixa Orellana*) collected in two communities in Cunduacán, Tabasco, Mexico.

These were taken to the Campus Tabasco del Colegio de Postgraduados where they were analyzed in the Animal Science Laboratory and Central Laboratory.

Physicochemical determination and bixin content of annatto seeds

The following parameters were determined for the samples: ashes (Kirk *et al.*, 1996), moisture (weight difference), dry matter (Nielsen, 2019), protein (Kjeldahl, AOAC, 1980), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest and Wine, 1967), fats (Soxhlet, 1990) and bixin extraction (Vázquez, 2001). They were performed on each of the original samples and a repeat for each determination. Of the eight samples collected, all were subjected to the seven determinations mentioned above. Except for dry matter, only two varieties were tested. This was due to the fact that when the collection was made, fresh material was needed, and the harvest date had already passed.

Ashes

The methodology developed by Kirk *et al.* (1996) was used. First, the percentage of organic matter (OM) was calculated with the following formula:

$$\%O.M \equiv \frac{DM - RW}{DM} \times 100$$

(*DM*) Dry Matter; (*RW*) Residual weight.

The percent of ashes was calculated with the following formula:

$$\%C \equiv 100 - \%O.M$$

%*C*: Percent of Ashes; %*O.M.*: Percent organic matter.

Moisture

Moisture determination was only carried out for samples 6 and 8.

$$\%DM = 100 - \%H$$

*P*1: Initial weight; *Pf*: Final weight.

Dry Matter

The methodology proposed by Nielsen (2019) was used.

$$\%H = \frac{P1 - Pf}{P1} \times 100$$

%*H*: Percent of moisture; %*DM*: Percent of Dry Matter.

Protein

The Kjeldahl method was used, and calculations were made using the following formulas:

$$\%P = \frac{(GTHCL)(NHCL)(1.4)}{Pm} \times 6.25$$

%*P*: Percent of Proteins; *GTHCL*: Total hydrochloric acid consumption; *NHCL*: Normality of hydrochloric acid; *Pm*: Sample weight.

Two adjustment factors were used: Adjustment factor for nitrogen (0.014 mill equivalents multiplied by 100 divided by final sample weight) and Adjustment factor for protein (0.0625 mill equivalents multiplied by 100).

Neutral Detergent Fiber (NDF)

Yields of recovered neutral detergent fiber were expressed as a percentage. Using the following formula:

$$\%FDN = \frac{(bag + sample) - (final\ weight - bag\ weight)}{sample\ weight} \times 100$$

Acid Detergent Fiber (ADF)

The yield calculations were made using the following formula:

$$\%FDA = \frac{(bag + sample) - (final\ weight - bag\ weight)}{sample\ weight} \times 100$$

Fats

The procedure was carried out with the following formula:

$$\%raw\ fat = \frac{M2 - M1}{M} \times 100$$

M: Sample weight; *M*1: flask weight; *M*2: flask weight with fat.

Bixin Extraction

The obtained absorbance was read and the bixin content is calculated using the following formula:

$$\%Bixin = \frac{\left[A_{500} + A_{404} - \left(0.256 \times A_{500}\right)\right]}{286.6 \times 1 \times a} \times 100$$

A=Absorbance of the test solution at the indicated wavelength; 1=Standard cell length (in cm); *a*=Sample concentration (in g/L); 286.6=Absorbance of bixin at 500 nm in chloroform (molar extinction coefficient); 0.256=Factor related to the absorbance of bixin in chloroform at 404 nm and 500 nm.

RESULTS AND DISCUSSION

The results obtained from the physicochemical characterization of the samples are presented in Table 1, where variations in the contents between samples are observed.

Regarding ashes content, sample M1 (7.18%) was higher than all other samples. While the lowest value was found in M6. For moisture content the highest value 41.26% was for M6 from the community of Yoloxochitl and the lowest moisture content in the seeds of sample M8 (33.55%) from Monte Grande. In terms of dry matter, the highest value

Table 1. Physicochemical characteristics of annatto seeds.

Samples	Ash	Moisture	Dry matter	Protein	NDF	FDA	Fats
	%						
M1	7.18	-	-	15.00	74.70	87.97	9.42
M2	4.61	-	-	14.28	65.48	81.03	13.80
M3	5.34	-	-	16.03	75.96	91.05	37.16
M4	5.52	-	-	15.58	65.20	80.46	24.41
M5	5.40	-	-	15.99	75.89	88.70	15.04
M6	3.72	41.26	58.73	15.03	58.66	91.10	23.91
M7	4.32	-	-	14.15	53.31	86.65	28.29
M8	3.84	33.55	66.45	15.13	65.46	90.22	28.29

Note: M1, M2, M3, M4, M6, M7=Samples taken from the community Yoloxochilt 3ª. Section. M5, M8=Samples taken from the Monte Grande community (Arias-Pérez and De Dios-Durán, 2013).

66.45% was obtained in M8 and the lowest in sample M6. On the other hand, sample M3 presented the highest value of 16.03% protein, followed by M5 (15.99%) and the sample with the lowest protein content was M7 with 14.15%. For Neutral Detergent Fiber (NDF) the highest value 75.96% was reached in sample M3 and the lowest value 53.31% in sample M7. For Acid Detergent Fiber (FDA) the highest value 91.10% was obtained in sample M6 and in sample M4 the lowest value with 80.46%. For fat content the highest value (37.16%) was obtained in sample M3 and the lowest value of 9.42% in sample M1.

The results reached in this research for the ash variable are similar to those achieved by authors such as Córdoba (1987), Jaramillo and Muñoz (1992), CNP (2001) and SDIC (2001), who, in studies carried out with annatto of the red variety with abundant filaments, found values that varied from 4.50 to 7.97%. For protein content, the results of the study were superior since the range varied from 14 to 16%, while the previously mentioned authors obtained ranges from 13.00 to 14.24%. The same authors found that moisture content ranged from 8 to 13%. Whereas in the study the two samples M6 and M8 presented moisture contents of 41.26 and 33.35%, respectively. Authors such as, Arias-Pérez and De Dios-Durán (2013) in the same variety of hearty red achiote without filaments obtained moisture percentages of 6.43%; this could be since the authors used very small samples of 0.2 g.

Values for NDF ranged from 53.31 to 75.96% and for FDA from 80.46 to 91.10%, which were higher than those obtained by Arias-Pérez and De Dios-Durán (2013), who reported an NDF content of 47.98% and FDA of 39.09%. As the FDA value increases, the digestibility of the seed is reduced as the cell wall is composed of cellulose and lignin (FOSS, 2018). Regarding fat content, the results showed that sample M3 had the highest value of 37.16%, while sample M1 had the lowest content of 9.42%. These results were higher than those achieved by Noguiera-Carvalho *et al.* (2010) who reported only 4.5% of ethereal fat in annatto seeds.

Table 2. Bixin extraction performed on annatto seeds and commercial paste.

Sample	Bixin (%)
Control	0.00
Annatto paste	0.56
M1	3.99
M2	4.09
M3	3.39
M4	3.86
M5	2.00
M6	3.74
M7	1.76
M8	1.08

Note: M1, M2, M3, M4, M6, M7= Samples taken from the community Yoloxochilt 3ª. Section. M5, M8= Samples taken from the community Monte Grande. Paste achiote= Sample taken from the annatto paste.

The results of this study differ from those obtained by Valadez-Villarreal *et al.* (2020), who achieved 8.2% bixin in annatto seeds when using an alkaline method for the extraction of the dye. The results varied according to the samples collected. The bixin content was higher (4.09%) in sample M2, the heartwood red variety without filaments, than in the other samples. In samples M5 (green annatto with abundant filaments), M7 (hearty red with few filaments) and M8 (hearty red with abundant filaments) the values found range between 1.08 and 2%, the lowest value corresponds to the annatto paste with 0.56% of bixin. This could be since this paste is commercial. INTITEC (2013), recommends for technical requirements in processing plants, that the concentration of bixin should not be less than 2.5%.

CONCLUSIONS

To be approved as an additive, a substance must be well characterized chemically and must pass the toxicological controls established by the corresponding health authorities. In addition, its need must be demonstrated in such a way that its use implies technological advantages and benefits for the consumer. Therefore, the annatto grown in rural communities in Tabasco can be an alternative as raw material of high nutritional value for the food industry and the sample with the highest bixin content should be recommended for its cultivation.

REFERENCES

Acero, G. 2007. Manual de prácticas de Bromatología. 2007. Disponible en: http://www.uaa.mx/centros/cca/MVZ/M/6/Manualdepracticas29-1528.pdf. Fecha de consulta 1 de marzo de 2020.

AOAC. 1980. Oficial Methods of Análisis of the Association of Official Analytical Chemists. Thirteenth edition. 353 p.

Arias-Pérez, I.M. y De Dios-Durán, F.M. 2013. Caracterización morfológica de una muestra local de Bixa orellana L., en Tabasco, México. *Revista Agroproductividad, 6*(10):91-97.

Beltrán, R.C.A. 2014. Diseño de un sistema de costos para una empresa agroindustrial de colorantes naturales-achiote. Trabajo de grado de maestría en Contabilidad con mención en Costos y Presupuestos en la Gestión Estratégica. Universidad Nacional Mayor de San Marcos. Facultad de Ciencias Contables. Lima, Perú. 142 pp.

Camacaro, J., Gómez, J., Jiménez, M., Vega C. y Manganiello L. 2018. A liposoluble colorant from Annatto seeds (*Bixa orellana* L.) as an input for food industry. *Revista Ingeniería UC, 2*(25):291-306.

Cordoba, J. A. 1987. El achiote, cultivo, beneficio y posibilidades de exportación. *Revista ESSO Agrícola*. Bogotá, Colombia. pp. 37.

CNP. 2001. Consejo Nacional de Producción. Desarrollo de Productos. Procesos Agroindustriales. Ficha Técnica de Información Nutricional de Achiote. http://www.mercanet.cnp.go.cr/nutriachiote.html.

FDA. 2001. Food and Drugs Administration. Summary of color additives listed for use United States in food, drugs, cosmetics and medical devices. URL: http://www.cfsan.fda.gob/~dms/opa-col2.html. Fecha de consulta, 22 de enero de 2022.

FOSS. 2018. El análisis de la fibra en el pienso animal Fibra cruda, fibra detergente neutra (FDN) y fibra detergente ácida (FDA) - los estándares y las opciones de automatización. Analytics Beyond Measure, e-book. 32 pp.

INTITEC. 2013. 3 Normas Técnicas Peruanas en su versión 2013sobre pinturas, identificación y tecnología nomenclatura de maderas comerciales y de especias y condimentos. Resolución Comisión de Normalización y Fiscalización de Barreras Comerciales no Arancelarias. No. 75-2013 CNB/INDECOPI. Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual. Lima, Perú.

Jaramillo, M. C. A. y Muñoz M. O. A. 1992. Extracción de colorantes de Achiote. Trabajo de Grado (ingeniero Químico). Medellín. Universidad de Medellín. Facultad de Mina. Departamento de Procesos Químicos. 100 pp.

Kirk. R.S., Egan H. y Sawyer, R. 1996. Composición y Análisis de Alimentos de Pearson. Editorial CECSA. Segunda edición. Grupo Patria Cultural S.A. DE C.V. ISBN 9682612640, 9789682612640. México, D.F. 777 pp.

La Química y la alimentación. 2018. Archivo 28. Foro permanente Química y Sociedad. Federación Empresarial de la Industria Química Española. Madrid, España. 20 pp. Disponible en www.quimicaysociedad.org

Nielsen, S.S. 2019. Food Analysis Laboratory Manual. Third Edition. Springer International Publishing, AG. Gewerbestrasse 11, 6330 Cham, Switzerland. 244 pp.

Nogueira-Carvalho, P.R., Gomes da Silva, M., Gomes Fabri, E., Da Rocha Tavares, P.E., Mello Martines, A. Spatti, L. 2010. Bixin and lipids content in annato seeds from collection of the Instituto Agronómico (IAC). Brangantia, Campinas, 519-524.

NOM-086-SSA1-1994. Norma Oficial Mexicana Bienes y Servicios. Alimentos y bebidas no alcohólicas con modificaciones en su composición. Especificaciones Nutrimentales. SEGOB. Diario Oficial de la Federación. DOF: 22/12/2010.

NOM-120-SSA1-1994. Norma Oficial Mexicana Bienes y Servicios. Prácticas de higiene y sanidad para el proceso de alimentos, bebidas no alcohólicas y alcohólicas. DOF: 28/08/1995.

NOM-131-SSA1-1995. Norma Oficial Mexicana, Bienes y servicios. Alimentos para lactantes y niños de corta edad. Disposiciones y especificaciones sanitarias y nutrimentales. 97 pp.

NOM-147-SSA1-1996. Norma Oficial Mexicana, Bienes y Servicios. Cereales y sus Productos. Harinas de cereales, sémolas o semolinas. Alimentos a base de cereales, de semillas comestibles, harinas, sémolas o semolinas o sus mezclas. Productos de panificación. Disposiciones y especificaciones sanitarias y nutrimentales. Fecha de Publicación: 10 de Diciembre de 1999. 25 pp

NOM-185-SSA1-2002. Norma Oficial Mexicana, Productos y servicios. Mantequilla, cremas, producto lácteo condensado azucarado, productos lácteos fermentados y acidificados, dulces a base de leche. Especificaciones sanitarias. DOF: 16/10/2002

NOM-213-SSA1-2002. Norma Oficial Mexicana, Productos y servicios. Productos cárnicos procesados. Especificaciones sanitarias. Métodos de prueba. DOF: 11/07/2005.

Rivera-Madrid, R. 2021. La cadena de valor del achiote (*Bixa orellana*) detrás del avance científico y tecnológico. Desde el Herbario CICY, 13: 222–226 (18/noviembre/2021). Centro de Investigación Científica de Yucatán, A.C. http://www.cicy.mx/sitios/desde_herbario/ISSN: 2395-8790

Sahaza, D.P. 2001. El Achiote. Disponible en: http://www.unalmed.edu.co/~crsequed/achiote.html. Fecha de consulta, 1 de febrero de 2023.

Salazar, G.A. 2014. Análisis Bromatológico. Disponible en: http://www.corpoica.org.co/sitioweb/Documento/JatrophaContrataciones/Analisisbromatologico.pdf. Fecha de consulta 28 de febrero de 2014.

SDIC (2001). Secretaría de Desarrollo Industrial y Comercial. Achiote. http://oaxaca.gob.mx/sedic/agronegocios/spanish/achiote.html. Fecha de consulta 13 de marzo de 2021.

Soxhlet, F. V. 1990. Official Methods of Analysis AOAC International. 21st Edition, U.S.A. 1-2 p.

A. Valadez-Villarreal, E. López-Hernández, F.L. Ruíz-Santiago, J.A. Hernández-Becerra, R. García-Jiménez. 2020. Aplicación de un extracto de achiote (*Bixa orellana*) en una formulación de un sistema modelo oleoso. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos*, 5:609-613.

Soest, P. J. y R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Assoc. Off. Agr. Chem. Journal*, 50:50-55.

Vázquez, H.C. 2001. Estudio preliminar de la degradación de bixina en polvo en los diferentes tipos de empaques y temperaturas establecidas. Tesis Licenciatura Instituto Tecnológico de Villahermosa. Villahermosa, Tabasco, México.