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

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ABSTRACT

Objective: The objective of this study was to carry out a bromatological and physicochemical analysis of local annatto seeds and a commercial paste.

Design/methodology/approach: The vegetal material used were local annatto seeds to which physicochemical analysis was carried out to determine ashes (Kirk *et al.*, 1996), moisture (weight difference), dry matter (Nielsen, 2019), proteins (Kjeldah, AOAC, 1980), FDA and FDN (Van Soest and Wine, 1967), fat (Soxhlet, 1990). In addition, bromatological analysis was carried out on the samples and the commercial paste (Vázquez, 2001).

Results: The results obtained from the physicochemical analysis varied in ranges, where the M3 sample (dark heart-shaped annatto without filaments) presented the highest values. Regarding the bixin content, the highest percentage with 4.09% was obtained in the M2 sample (heart-shaped red annatto without filaments) and the commercial paste was the lowest with 0.56%.

Limitations on study/implications: The importance of carrying out the bromatological and physicochemical analysis of the local annatto seeds was to determine which of the local samples and the commercial paste contain the highest amount of bixin. The antioxidant properties of bixin, together with a long shelf life in addition to its well-established safety characteristics, result in a very promising natural coloring agent in the food industry, given that it is an FDA-approved food additive: E160 b.

Findings/conclusions: The physicochemical analyzes varied in ranges, where the M3 sample (dark heart-shaped annatto without filaments) presented the highest values. The highest bixin content was found in the smooth heartwood red variety and the lowest value in the commercial paste.

Keywords: Accessions, food, proximal analysis, bixin.

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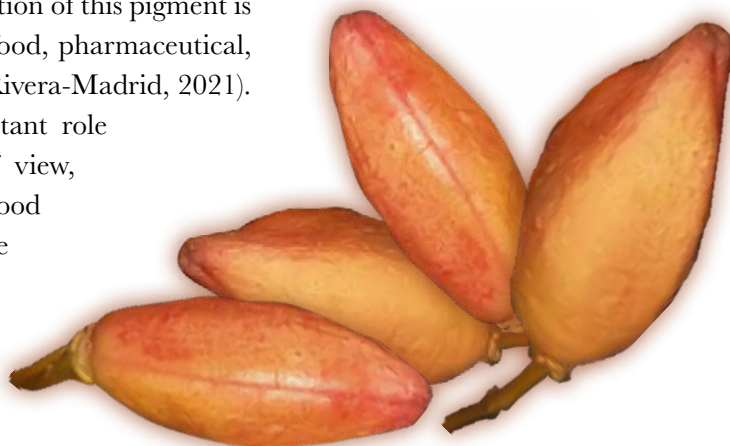
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INTRODUCTION

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. The 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 also 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 also a commercial paste.

MATERIALS AND METHODS

Annatto seed samples (Figure 1) collected in two communities in the municipality of Cunduacán, Tabasco, Mexico, were used as plant material: six corresponded to the Yoloxochitl 3rd section community and two samples to Monte Grande. The samples were



Figure 1. Annatto samples and seeds collected in two communities in Cunduacán, Tabasco.

placed in paper bags with a capacity of 500 g and labeled; each sample weighed approximately 200 g. 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; *%OM*: Percent of organic matter.

Moisture

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

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

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

Dry Matter

The methodology proposed by Nielsen (2019) was used.

$$\%H = \frac{P_1 - P_f}{P_1} \times 100$$

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

Protein

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

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

%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:

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

Acid Detergent Fiber (ADF)

The yield calculations were made using the following formula:

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

Fats

The procedure was carried out with the following formula:

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

M: Sample weight; *M1*: flask weight; *M2*: flask weight with fat.

Bixin Extraction

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

$$\%Bixin = \frac{[A500 + A404 - (0.256 \times A500)]}{286.6 \times l \times a} \times 100$$

A=Absorbance of the test solution at the indicated wavelength; *l*=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 66.45% was obtained in M8 and the lowest in sample M6. On the other hand, sample M3

Table 1. Physicochemical characteristics of annatto seeds from two communities in the municipality of Cunduacán, Tabasco, Mexico.

Samples	Ashes	Moisture	Dry Matter	Protein	NDF	ADF	Fats
	%						
S1	7.18	-	-	15.00	74.70	87.97	9.42
S2	4.61	-	-	14.28	65.48	81.03	13.80
S3	5.34	-	-	16.03	75.96	91.05	37.16
S4	5.52	-	-	15.58	65.20	80.46	24.41
S5	5.40	-	-	15.99	75.89	88.70	15.04
S6	3.72	41.26	58.73	15.03	58.66	91.10	23.91
S7	4.32	-	-	14.15	53.31	86.65	28.29
S8	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 Yoloxochitl 3^a. Section. M5, M8=Samples taken from the Monte Grande community (Arias-Pérez and De Dios-Durán, 2013).

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 due to the fact that 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 Nogueira-Carvalho *et al.* (2010) who reported only 4.5% of ethereal fat in annatto seeds.

Table 2 shows the results obtained in the extraction of bixin and commercial annatto paste.

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 3^a. 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 (heartly red with few filaments) and M8 (heartly 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 due to the fact that 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.

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