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# CHEMICAL, NUTRITIONAL, AND BIOLOGICAL COMPOSITION OF THREE SEED MORPHOTYPES OF *BIXA ORELLANA* L. BIXACEAE (ACHIOTE) IN THE YUCATAN PENINSULA, MEXICO

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**Abstract**

*Bixa orellana* L. is endemic to the tropical zone of the Americas. In a previous study, our research group detected anti-inflammatory properties in the leaves of three accessions of *Bixa orellana* L. in the Maya area. Our next objective was to perform a morphological analysis of the seeds and determine their chemical and nutrient composition, and the results are described herein. The morphological characterization was based on 8 quantitative and 14 qualitative characters, and some important similarities and differences were found in the length and shape of leaves and seeds, ramifications, dehiscence of fruits, and number of seeds per fruit. Regarding the chemical composition, accession 3 had the highest values for bixin (40.83 ± 1.27 mg/g), phenolic compounds (9.65 ± 0.18 mg GAE /g milligrams gallic acid equivalents per gram of seeds, carbohydrates (43.3 ± 0.24%), and functional dietary fiber, while accession 1 had the highest values for proteins (13.83 ± 0.04%) and ash (4.53 ± 0.2%). The plants of accession 3 whose seeds had the highest phenolic compounds and bixin content also had the leaves with the greatest anti-inflammatory effect. Thus, the plants of accession 3 are an ideal candidate for the propagation and integral use of their biological material. The next challenges are the study of the annatto plant's reproduction and the chemical composition of its fruits.

**Key words:** *Bixa orellana*, Bixin, Proximal and chemical composition, Dietary fiber.

## Introduction

Currently, there is great interest in the applications of different plants in the food and other industries with reference to their potential nutritional, antioxidant, and pharmacological properties and economic benefits (Pierpaoli *et al.*, 2013). One of these plants is *Bixa orellana*, known as annatto or achiote in Mexico. It is a tropical tree belonging to the *Bixaceae* family, native to the Americas and is considered one of the most important species in Maya-Yucatecan orchards. It has a high commercial value and national and international demand stemming from the use of a compound found in its seeds (bixin) as a natural colorant. In addition, it is used in local cuisine and in traditional medicine across Mexico and Central and South America for the treatment of various ailments and diseases. Its beneficial effects may be related with the presence of secondary metabolites in different tissues (Zarza-García *et al.*, 2017). Raga *et al.*, (2011) found that these plants contain bioactive compounds (sesquiterpene) that are effective in the treatment or prevention of pain and other conditions such as inflammation in relation to various ailments. Also, our research group previously studied three accessions of annatto and found that the leaves of morphotype 3 showed anti-inflammatory activity similar to indomethacin (Zarza-García *et al.*, 2017).

Several studies have characterized the chemical, biological, and antioxidant contents of the annatto plant, and also investigated propagation methods to facilitate its cultivation as well as the production of bixin year-round. However, its seeds, seedlings, and plants have long been exchanged and propagated across the Maya-Yucatecan region, so there is a wide diversity of morphotypes.

In this background, we aimed to study the morphological composition of three morphotypes of annatto seeds according to 8 quantitative and 14 qualitative characters and to perform a chemical and nutrient analysis in order to determine the moisture, ash, protein, carbohydrate, total phenol, fiber, and functional fiber contents in addition to the antioxidant capacity. It was also aimed to correlate the anti-inflammatory effect previously found in annatto leaves with the seed characteristics. These results contribute to the characterization of the different morphotypes of annatto and support the integral use of this plant and the reproduction of its biological material.

## Materials and Methods

**Materials:** The study examined the seeds of three accessions of *B. orellana* L. (Fig. 1) labeled with the numbers 8, 9, and 11 (according to Accession/Voucher), which were renamed herein as 1, 2 and 3 (Zarza-García *et al.*, 2017). Seeds were collected from the plants of the Achiote Network of the Technological Institute of Conkal, Mexico, a part of the National System of Plant Genetic Resources for Food and Agriculture (SINAREFI).

**Morphological analysis and characterization**: The morphological parameters of plants freshly harvested from different sites (accessions) were determined (Fig. 1). The plants were marked and referenced by geolocation using GPS.

A total of 8 quantitative characters (total tree height, first branch height, trunk diameter, leaf blade length, leaf width, petiole length, fruit length, fruit width) and 14 qualitative characters (trunk surface, number of fruits in each plant, seed form, number of seeds of each fruit) were assessed. The parameters were measured in each corresponding part of the plant and were mainly related with the vegetative features, leaves, and fruits.

Tree height was determined using an inclinometer (Suunto, Finland). A measuring tape was used to determine the height of the first branch and trunk diameter at 0.10 m from the ground. Leaf length and width and fruit length were measured using Vernier calipers. The qualitative parameters were assessed by visual observation and subjective interpretations and compared to the characters established by the International Plant Genetic Resources Institute for avocado (*Persea* spp.) due to the absence of data on *B. orellana* L.

**Seed preparation:** The seeds were depigmented using a standardized technique. Five grams of seeds were weighed, and 150 mL of ethyl acetate was added. An extraction was carried out for 3 h in a Soxhlet apparatus. The seeds were filtered and recovered and then dried for 40 min in an oven (40°C). Subsequently, chloroform (30 mL) was added, and the seeds were stirred at 60°C. Then, they were rinsed several times until they were depigmented. The seeds were dried again and placed in an oven (60°C) to volatilize the solvent and, afterwards, were crushed using a mortar. The crushed seeds were then ground with an electric coffee grinder for nuts and spices (model CG-8120, ANMER, 200 watts) with stainless steel blades until a powder was obtained.

**Proximal chemical quantification of seeds:** The proximate analyses were performed in triplicate according to the method proposed by the Anon., (2005) for moisture content, ash, proteins (nitrogen), and ethereal extraction.

**Determination of carbohydrates**: The phenol-sulfuric acid method of Dubois *et al.*, (1956) was used to determine the carbohydrate content. One gram of seed powder of each accession (depigmented) was weighed, dissolved in 50 mL of water in a test tube, boiled at 90°C for 10 min, starch solubilized, liquefied (homogenized), centrifuged, and filtered. The extract was stored in a tube, and 0.25 mL of 5% phenol and 1.25 mL of concentrated $H_2SO_4$ were added. An aliquot of 25 µL was taken from each extract and adjusted to 5000 µL (from each accession). From this dilution, the following reaction was prepared: 1 mL of the previous solution of each accession was added with 500 µL (0.5 mL) of phenol and placed in cold water. Then, 2.5 mL of concentrated $H_2SO_4$ was added, and the mixture was left to rest for 15 min. The absorbance was read at 490 nm on an Agilent UV Visible 8453 spectrometer (Dubois *et al*., 1956).

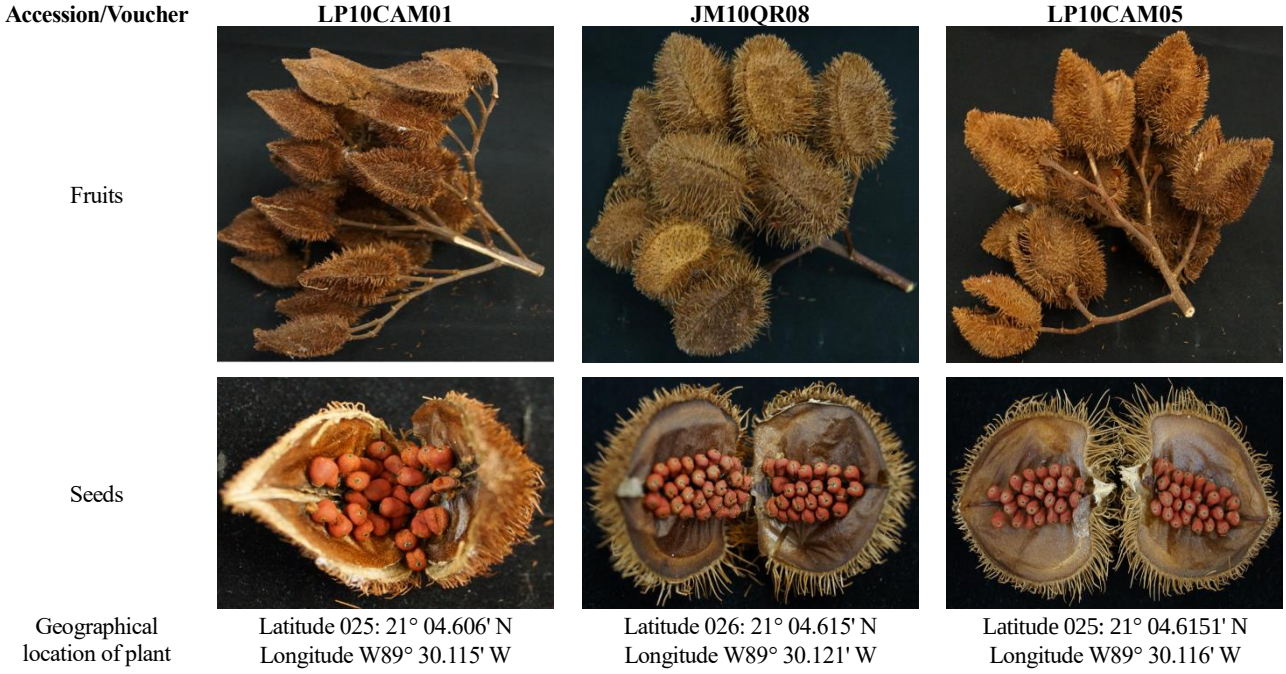


Fig. 1. Accession numbers, fruits and seed shapes, and georeferenced locations of three *Bixa orellana* L. accessions.

**Quantification of total dietary fiber, soluble fiber, and insoluble fiber:** The enzymatic-gravimetric method of Prosky *et al.*, (1988) was used to determine the dietary fiber (Barbosa-Martín *et al.*, 2016). One-half gram of celite and 10 mL of 78% ethanol were added to four crucibles for dietary fiber determination. These were then dried at 130 °C for 90 min in an oven until a constant weight is achieved (Fisher Scientific), left to cool in a desiccator, and weighed. In quadruplicate, 1 g of seed flour of each accession was weighed, placed in an Erlenmeyer flask with 50 mL of 0.05 N phosphate buffer pH 6 (9.6593 g of $NaH_2PO_4$ and 1.4 g of $Na_2HPO_4$ [dibasic]) and diluted in 700 mL of distilled water. The pH was adjusted to 6 and the volume to 1 L. Then, the flasks were placed in a water bath (New Brunswick R76) at 90 °C with constant stirring at 60 rpm for 10 min and, without removing them, 0.1 ml (100 µL) of thermostable α-amylase (Sigma A-3306) was added. The samples were covered and stirred continuously for an additional 15 min.

The flasks were cooled, and the pH was measured and adjusted to 7.5 with 0.0275 N NaOH using a potentiometer (Thermo Scientific Orion Star A215). Subsequently, they were placed in a bath at 60°C for 10 min and, without removing them, 0.1 mL of protease (Sigma P-3910) (0.025 g of protease dissolved in 0.5 mL of phosphate buffer pH 6) was added. They were then covered and stirred for 30 min at 60 rpm.

After cooling, the pH was adjusted to 4 with 0.325 N HCl. and were placed again in a bath at 60°C for 10 min with stirring. Without removing them, 0.3 mL of amyloglucosidase (Sigma A-9913) was added, and they were stirred for another 30 min. After digestion, 95% ethanol preheated to 60°C was added to each flask in a ratio of 1:4 (v/v). This step was only performed for the flasks used to determine total fiber but omitted for the flasks used to determine soluble fiber. The content of each flask was washed and filtered under a vacuum in a previously prepared crucible. The residue recovered in each crucible was washed with 20 mL of 78% ethanol, two 10-mL portions of 95% ethanol, and two 10-mL portions of acetone.

Finally, the crucibles with residues were dried at 105°C for 12 h in an oven, cooled in a desiccator, and weighed (P1). The residues of two crucibles were used to determine crude protein (P2); they were scraped and placed in aluminum to determine proteins. To determine ash content, the remaining two residues were incinerated at 550 °C for 4 h and were then cooled and weighed (P3). For the quantification of total dietary fiber (TDF), the following equation was used:

$$\% \text{ TDF} = (P1 - P2 - P3) / \text{sample weight.}$$

For the quantification of insoluble dietary fiber (IDF), the same procedure as for TDF was performed, but omitting the final treatment with 300 mL of preheated 95% ethanol (digestion). The percentage of soluble dietary fiber (SDF) was obtained from the difference between TDF and IDF. The filters must be completely clean, so they were washed with distilled water and placed in the oven at 100°C for 10 min and then in the muffle at 550°C for 1.5 h. Then, they were placed under constant weight in an oven for 2 h at 120°C, removed, cooled, and weighed. The flasks with a smaller volume had insoluble fiber. The flasks with a larger volume with floating mucus had total fiber.

**Water retention capacity:** The water retention capacity (WRC) was determined according to the methodology of Chau *et al.*, (1997) with some modifications of Segura-Campos *et al.*, (2014). One gram of seed powder of each accession was weighed in triplicate. Twenty mL of distilled water were added, subsequently stirred for 1 min in a vortex, and centrifuged at 2250 xg for 30 min at 25°C. The volume of the supernatant was measured with a graduated cylinder, and the WRC was expressed as grams absorbed per grams of sample.

**Oil retention capacity:** The oil retention capacity (ORC) was measured according to Chau *et al.*, (1997). In triplicate, 10 mL of saturated corn oil was added to 1 g of each accession. The mixture was stirred for 1 min in a vortex and then centrifuged at 2200 xg for 30 min at 25°C. The volume of the supernatant was measured with a graduated cylinder, and the ORC was measured as follows: ORC = grams of retained oil (volume of supernatant × 0.89 g/ml, density of oil) per grams of sample (Segura-Campos *et al*., 2014).

**Absorption capacity of organic molecules**: The absorption capacity of organic molecules (ACOM) was measured according to Zambrano *et al.*, (2001). In a 50-mL centrifuge tube, 3 g of dried sample was placed in an excess of corn oil (approx. 10 mL) for 24 h at 25°C. Afterwards, it was centrifuged at 2000 xg for 15 min at 25°C (Beckman GS-15R). The ACOM was expressed as the absorbed hydrophobic components and calculated as the weight gain of the sample (g of absorbed oil/g of sample) (Barbosa-Martín *et al.*, 2016).

**Total phenolic compounds of methanol and ethyl acetate extracts:** Total phenolic compounds (TPCs) were quantified in triplicate according to the Singleton & Rossi (1965) method adapted by Aarland *et al.*, (2017) and Castillo-López *et al.*, 2017.

**Determination of *in vitro* antioxidant activity (ABTS $^{+}$)**: Antioxidant activity was measured according to the Re *et al.*, (1999) method adapted by Aarland *et al.*, (2017) and Haddad *et al*, (2019)

**Determination of bixin content by high performance liquid chromatography (HPLC):** The identification and quantification of bixin was performed using the method described by Fraser *et al.*, (2000) with the slight modifications of Raddatz Mota *et al.*, (2016).

**Statistical analyses**: One-way ANOVAs were performed followed by TUKEY multiple comparison tests. Values were considered statistically significant at $p<0.05$. The test was performed in the Stat-graphics Centurion XVI software (*version* 16.1.18).

## Results and Discussion

**Morphological characteristics:** The vegetative, foliar, and fruit morphological characteristics of the three *B. orellana* L. accessions are presented in Table 1. The detailed similarities and differences are following:

a) Vegetative features. Differences were observed in total tree height (2.23 to 3.10 m), first branch height (0.11 to 0.18 m), and trunk diameter (0.07 to 0.45 m).
b) Leaf features. Similarities were observed in leaf length (0.137–0.200 m) and width (0.090–0.071 m). The length of the petiole ranged from 0.034–0.044 m. No differences were observed in the shape of the leaf base; all accessions had a truncated form. The color of the central vein varied from green/yellow to green (Table 1).
c) Fruit features. The shape of the fruit varied at the base, being flat or ovoid. Accessions 2 and 3 showed dehiscence, while accession 1 presented indehiscence. Variation in the length and width of fruits was observed. No difference in the apex and shape of the base (globular and flat) was observed. The shape of the cluster was irregular in all three accessions. High variability was observed in the number of clusters per plant (from 80 to 130), number of fruits per cluster (from 8 to 17), and number of fruits per plant (between 640 and 2210). Differences in the shape and number of seeds were also observed. Accessions 1 and 3 had an ovoid shape and accession 2 a triangular shape. The number of seeds per fruit varied from 28 to 50.

In conclusion, the three studied achiote accessions presented some important similarities and differences in the leaf and seed length, ramifications, leaf and seed shape, dehiscence of fruits (an important aspect related to bixin content), number of clusters, number of seeds per fruit, and shape capsule.

**Proximal chemical composition:** The proximal chemical composition of the seeds of the three *B. orellana* L. accessions is presented in Table 2. Accession 3 contained a higher protein and fat content, while the ash content was higher in accession 2. These values are within the ranges reported for other seed accessions of the same geographical area (Valério *et al.*, 2015; Dike *et al.*, 2016).

In addition, these results confirm the bromatological characteristics of interest, such as the percentages of protein and carbohydrate. These data provide information on the properties and macronutrients of the seeds of the three accessions, which can be subjected to more specific studies in the future to determine the presence of additional compounds or nutrients.

**Table 1. Vegetative, foliar, and fruit features of three Mexican accessions of *Bixa orellana* L.**

| | Accessions | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| | **LP10CAM01** | **PC10YUC08** | **LP10CAM05** |
| **a. Voucher number** | | | |
| **b. Vegetative features** | | | |
| Total length (m) | 2.23 | 2.80 | 3.10 |
| Height of first branch (m) | 0.18 | 0.14 | 0.11 |
| Trunk diameter (m) | 0.45 | 0.07 | 0.13 |
| Trunk surface | Smooth | Smooth | Smooth |
| Sap color | Absent | Absent | Absent |
| **c. Foliar features** | | | |
| Leaf length (m) | 0.137 | 0.142 | 0.200 |
| Leaf width (m) | 0.090 | 0.071 | 0.080 |
| Leaf base shape | Truncated | Truncated | Truncated |
| Petiole length (m) | 0.034 | 0.040 | 0.044 |
| Central vein color | Green/yellow | Green/yellow | Green |
| **d. Fruit features** | | | |
| Shape | Flattened base | Ovoid | Ovoid |
| Dehiscence | Indehiscent | Dehiscent | Dehiscent |
| Length (m) | 0.035 | 0.045 | 0.040 |
| Width (m) | 0.040 | 0.034 | 0.039 |
| Apex shape | Globular | Globular | Globular |
| Cluster shape | Irregular | Irregular | Irregular |
| N° clusters per plant | 90 | 80 | 130 |
| N° of fruits per plant | 640 | 1280 | 2210 |
| Seed shape | Ovoid | Triangular | Ovoid |
| N°of seeds per fruit | 28 | 50 | 50 |

[*] Germplasm Bank of the Technological Institute of Conkal, Yucatán. All measurements are given as means. Abbreviations: m: meters

**Table 2. Chemical and nutritional composition of the accessions.**

| | Accessions | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| | **LP10CAM01** | **PC10YUC08** | **LP10CAM05** |
| **a. Chemical/proximal** | | | |
| % Proteins | $13.83 \pm 0.04^{b}$ | $14.25 \pm 0.14^{b}$ | $12.24 \pm 0.05^{a}$ |
| % Lipids | $1.09 \pm 0.03^{a}$ | $1.46 \pm 0.03^{b}$ | $3.19 \pm 0.07^{c}$ |
| % Ash | $4.53 \pm 0.2^{b}$ | $5.48 \pm 0.04^{b}$ | $5.57 \pm 0.06^{a}$ |
| % Total carbohydrates | $11.85 \pm 0.07^{a}$ | $27.51 \pm 0.04^{b}$ | $43.3 \pm 0.24^{c}$ |
| Bixin (mg/g) | $20.18 \pm 0.77^{a}$ | $36.91 \pm 2.52^{b}$ | $40.83 \pm 1.27^{c}$ |
| **b. Dietary fiber (%)** | | | |
| TDF | $53.091 \pm 0.6607^{a}$ | $65.697 \pm 1.5076^{c}$ | $61.062 \pm 2.5338^{bc}$ |
| IDF | $42.357 \pm 1.9595^{a}$ | $51.836 \pm 1.66594^{c}$ | $47.117 \pm 1.44308^{b}$ |
| SDF | $10.734 \pm 1.775^{b}$ | $13.861 \pm 1.980^{c}$ | $13.945 \pm 2.526^{c}$ |
| **c. Functional fiber (%)** | | | |
| WRC | $3.23914 \pm 0.355095^{bc}$ | $2.49343 \pm 0.00137009^{a}$ | $3.74287 \pm 0.360243^{c}$ |
| ORC | $6.8054 \pm 0.128813^{a}$ | $7.25801 \pm 0.154323^{ab}$ | $7.37154 \pm 0.108445^{ab}$ |
| ACOM | $0.747668 \pm 0.0334534^{c}$ | $0.606252 \pm 0.00781378^{b}$ | $0.501679 \pm 0.0266986^{a}$ |
| **d. TPC and antioxidant capacity** | | | |
| $TPC_{(EAG, methanol)}$ | $5.66 \pm 0.32^{b}$ | $4.99 \pm 0.14^{a}$ | $9.65 \pm 0.18^{c}$ |
| $TEAC_{(ABTS+, methanol)}$ | $1.89 \pm 0.07^{a}$ | $2.43 \pm 0.01^{b}$ | $2.45 \pm 0.00^{b}$ |
| $TPC_{(EAG, ethyl acetate)}$ | $4.51 \pm 0.26^{a}$ | $6.84 \pm 0.45^{b}$ | $8.14 \pm 0.31^{c}$ |
| $TEAC_{(ABTS+, ethyl acetate)}$ | $2.20 \pm 0.09^{bc}$ | $1.46 \pm 0.18^{a}$ | $2.24 \pm 0.15^{c}$ |

Different letters in the same column indicate significant differences ($p<0.05$)

**Key: TDF,** Total dietary fiber; **IDF,** Insoluble dietary fiber; **SDF,** Soluble dietary fiber; **WRC,** Water retention capacity; **ORC,** Oil retention capacity. **ACOM,** Absorption capacity of organic molecules. **TPC,** Total phenolic content. **TEAC,** Trolox equivalent antioxidant capacity

**Total dietary (TDF), soluble fiber (SDF), and insoluble fiber (IDF):** In the literature, no reports on the dietary fiber content of annatto seeds were found (only the raw fiber content), so the present study is the first contribution to the subject. Dike *et al.*, (2016) reported that the raw fiber content of seeds of other varieties of annatto was 53.31% ± 0.07%. Raw fiber is considered to provide an approximate measure, mainly for structural polysaccharides and lignin (Mišurcová *et al.*, 2012); however, these data do not completely represent the total fiber content (least digestible part of food). The results obtained through the gravimetric enzymatic method described by Prosky *et al.*, (1988) and used herein are more specific because the polysaccharides are hydrolyzed with enzymes, allowing for the quantification of the proportion of soluble and insoluble fiber.

Table 2 shows the high percentage of TDF in the seeds of the three accessions, which is comparable to that of other species, such as chia (*Salvia hispanica* L.) and flaxseed (*Linum usitatissimum*) (Jiménez *et al.*, (2013), although dissimilar to that of rosehip seed (*Rosa rubiginosa*) (Jiménez *et al.*, 2013). Of these, annatto seeds have the highest TDF percentage, with the highest TDF being found in accession 2 (65.697% ± 1.5076%) and accession 3 (61.062% ± 2.5338%) and the lowest in accession 1 (53.091% ± 0.6607%).

The IDF of the seeds was higher than that reported for avocado seed (Barbosa-Martín *et al.*, 2016), whereas chickpeas (*Cicer arietinum*), beans (*Phaseolus* spp.), lentils (*Lens culinaris* L.), and peas (*Pisum sativum*) were found to have lower concentrations according to Tosh & Yada. (2010). This gives added value and functional importance to the studied annatto seeds as a source of insoluble fiber, which can increase intestinal motility. In particular, the highest percentage of SDF was found in accession 3 (13.945% ± 2.526%) and accession 2 (13.861% ± 1.980%) (Table 2).

The SDF of accession 1 (10.734% ± 1.775%) coincided with that of avocado seeds (Barbosa-Martín *et al.*, (2016). Meanwhile, accessions 2 and 3 (13.861% ± 1.980% and 13.945% ± 2.526%, respectively) had a higher SDF than avocado seeds (Barbosa-Martín *et al.*, 2016). This property is interesting given the physicochemical properties of SDF (gums, pectins, psyllium, and glucans), which are related to the physiological properties. In particular, SDF influences metabolic activity, absorbs water, and favors an increase in viscosity, which benefits gel formation and thereby aids in digestion and lubrication of feces and promotes a reduction in glycemia and plasma cholesterol (Slavin, 2005).

The highest percentage of TDF was observed in accession 2 (65.697% ± 1.5076%) and accession 3 (61.062% ± 2.5338%). These percentages are significantly similar to other plants considered to be good sources of total fiber, such as oats, which contain β-glucan (Jacometti *et al.*, 2015; Sterna *et al.*, 2015). Accession 1 (53.09% ± 0.6607%) had the lowest percentage of TDF, similar to that of dry beans (*P. vulgaris*), as reported by Tosh & Yada. (2010).

In conclusion, the total, soluble, and insoluble dietary fiber composition of the seed flour of the three accessions showed significant differences ($p<0.05$, confidence level of 95%). However, these values are within those previously reported for other seeds or legumes considered high in fiber (Table 2).

**Functional properties of dietary fiber:** The functional effects of fiber in the body are related to the physicochemical properties, type of fiber, amount ingested, and environmental conditions of the gastrointestinal tract. It is important to determine the functional properties of dietary fiber given its uses and applications in the food industry and human food (Yangilar, 2013). The seeds of accession 3 (Table 2) showed the highest percentage of water retention (3.74% ± 0.36), followed by those of accession 1 (3.23914% ± 0.355095) and accession 2 (2.494% ± 0.00137009), which showed the lowest water retention capacity. The oil retention capacity of the seeds of the three studied accessions (Table 2) showed no significant differences.

The absorption capacity of organic molecules (ACOM) showed significant differences ($p<0.05$) among the three accessions: The seeds of accession 1 (0.747668 ± 0.0334534) and accession 2 (0.606252 ± 0.00781378) had the highest ACOM.

In conclusion, the functional potential of the seed flour of accessions 2 and 3 was confirmed herein. This is due to their high content of soluble and insoluble fiber. These results also confirm the potential uses of annatto seeds: Their fibrous residues can function as potential nutraceuticals in the food (bread flour) and health industries.

***In vitro* antioxidant activity by ABTS of methanolic and ethyl acetate extracts:** The highest antioxidant activity was found in the methanolic extracts of accession 3 (2.45 ± 0.00 mM/g CAET) and accession 2 (2.42 ± 0.01 mM/g CAET). A similar trend was maintained when using ethyl acetate as the extraction medium (Table 2). The antioxidant activity found in this study is similar to that reported by Raddatz Mota *et al.*, (2016).

**Determination of bixin by HPLC:** The bixin content is presented in Table 2, with accession 3 having the highest level. The values coincide with the range (0.26–311 mg/g) previously reported by several authors (Viuda-Martos *et al.*, 2012; Raddatz Mota *et al.*, 2016). The bixin content is an important factor for commercialization, as a content of at least 2.7% is required.

## Conclusions

The results of the present study indicate that the seeds of the studied *B. orellana* L. accessions have functional potential because of their antioxidant, bromatological, and nutritional characteristics and, consequently, have beneficial uses and applications in the food industry and pharmaceutical industries.

In particular, the seeds of accessions 2 and 3 had the highest content of bixin. The seeds of accession 3 had the highest content of phenolic compounds and fiber and antioxidant activity. The seeds of accessions 1 and 2 had the highest protein content (13.83% and 14.25, respectively), whereas those of accession 3 had the highest total carbohydrate content (43.3%). In addition, the leaves of accession 3 showed anti-inflammatory activity like indomethacin, increasing the potential pharmaceutical value of this cultivar. Future studies should explore methods for the propagation of these accessions.

From an economic and functional perspective, accessions 2 and 3 are good options for the propagation of annatto in southeastern Mexico. Finally, considering the health benefits of dietary fiber (laxative and cholesterol and glucose attenuation), the seeds of accession 3 can function as a source of dietary fiber or be used in the food industry for the development of nutraceuticals and other functional food products.

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