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Effect of sonication on the content of bixin, norbixin, total phenols and antioxidant activity of extracts of five achiote accessions

Efecto de la sonicación en el contenido de bixina, norbixina, fenoles totales y actividad antioxidante de extractos de cinco accesiones de achiote

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Abstract

Pigments obtained from achiote seeds are highly demanded worldwide, being the bixin the second most important natural pigment. Due to this, the study of methods and conditions that allow to improve traditional processes for their extraction from achiote seeds is of particular interest. In some works, the functional activity of the obtained extracts is also evaluated. In this work we study the effect of a sonication treatment on the extraction of functional compounds of achiote seeds, as well as the antioxidant activity of such extracts, in comparison to a traditional agitation extraction. Sonication has shown some advantages such as being simple to apply, reducing extraction times and increasing extraction yields. The comparisons were made in hydrophilic and hydrophobic extracts obtained from 5 accessions of achiote from Yucatan Peninsula, Mexico. When applying sonication, time of extraction was reduced (33%), the content of polyphenols in the hydrophilic phases was increased (18-25%) as well as the norbixin content in the alkaline extracts (70-85%). A similar behavior was observed in the antioxidant activity of the hydrophilic (18-22%) and hydrophobic (10-20%) phases; however, the sonication had no effect on the content of bixin, the latter being the main product of commercial interest. These results suggest that some of the studied accessions had the potential to be used commercially and that sonication extraction can be a valuable alternative for obtaining extracts with higher contents of functional compounds or enhanced norbixin contents from achiote seeds.

Keywords: *Bixa orellana*; sonication; bixin; norbixin; antioxidant activity.

Resumen

Los pigmentos obtenidos de las semillas de achiote tienen una gran demanda mundial, siendo la bixina el segundo pigmento natural de mayor importancia. Debido a esto es de interés el estudio de métodos y condiciones que permitan mejorar los procesos tradicionales para su extracción a partir de semillas de achiote. En ocasiones, la actividad funcional de los extractos obtenidos también es evaluada. En este trabajo se estudia el efecto de un tratamiento de sonicación en la extracción de compuestos funcionales de semillas de achiote, así como la actividad antioxidante de dichos extractos, en comparación con un tratamiento de extracción tradicional por agitación. La sonicación ha mostrado tener ventajas como ser sencilla de aplicar, disminuir los tiempos de extracción e incrementar los rendimientos de extracción. Las comparaciones se realizan en extractos hidrofílicos e hidrofóbicos obtenidos de 5 accesiones de achiote provenientes de la península de Yucatán, México. Al aplicar el tratamiento de sonicación, el tiempo de extracción se redujo (33%), se incrementó el contenido de polifenoles (18-25%) y de norbixina (70-85%) en los extractos, así como la actividad antioxidante de los extractos hidrofílicos (18-22%) y de los extractos hidrofóbicos (10-20%); sin embargo, las sonicación no tuvo efecto en el contenido de bixina, el principal producto de interés comercial. Estos resultados apuntan a que algunas de las accesiones aquí estudiadas tienen el potencial de ser aprovechables comercialmente y que la extracción mediante sonicación puede ser una alternativa valiosa para la obtención de extractos con mayor contenido de compuestos funcionales o extractos con altos contenidos de norbixina a partir de semillas de achiote.

Palabras clave: *Bixa orellana*; sonicación; bixina; norbixina; actividad antioxidante.

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1 Introduction

Achiote (*Bixa orellana*) is a shrub native to tropical America. It is widely cultivated in tropical climate countries around the world for its use as a condiment as well as being acknowledged as one of the natural main sources of carotenoid pigments (Scotter, 2009). Current world production is estimated at about 14,500 tons, with Latin America accounting for about 60% of total production. Peru, Brazil and Mexico are among the leading producers (Smith, 2006; SNICS, SAGARPA, 2018). In Mexico, the Yucatan Peninsula is the main producer of achiote. Bixin and norbixin are the two major carotenoids in achiote seeds. Bixin content represents up to 90% of total carotenoids (Giorgi *et al*., 2013) and together with norbixin, commonly represent between 2-5% of total seed weight. Bixin is a one-tail methoxylated derivative of norbixin; the latter having carboxyl groups at both tails. It is usually mentioned that norbixin is the water-soluble form of the pigment, however, its solubility is low at neutral pH values; the presence of alkaline substances such as sodium or potassium hydroxides leads to ionizing modifications which facilitate its solubility (Albuquerque and Meireles, 2011; Giorgi *et al*., 2013). Bixin extraction is commonly performed with water (obtaining low yields) or with organic solvents, while norbixin is extracted in alkaline solutions. Both pigments find a wide variety of applications in different industries due to their safety, dyeing power, shades produced, as well as versatility (Smith, 2006; Scotter, 2009).

Methodologies that implicate less impact on the environment, lower resource consumption and low risk to operators and consumers are one of the technological fields that demand greater attention (Vilkhu *et al*., 2008). Some non-conventional methods are applied in order to increase both effectiveness and efficiency of the extraction methods. Among these new technologies, ultrasound extraction is particularly highlighted for its improvement in extraction efficiency, low operating costs, simplicity of application and extraction time reduction (Zuorro *et al*., 2011; Ruiz-Montañez *et al*., 2014). It is based on the cavitation process which implies the creation, growth and implosion of micro-bubbles causing the disruption of the cell wall and increasing temperature, facilitating the release of the target compounds and enhancing mass transport (Martínez *et al*., 2019; Yang *et al*., 2013). Sonication has been applied to obtain achiote seed extracts; however, the effect of sonication on the main components on achiote seed extracts (bixin, norbixin and total phenolic compounds) and on the antioxidant activity of these extracts compared with a traditional extraction method has not been reported so far (Cardarelli *et al*., 2008; Giorgi *et al*., 2013; Yolmeh *et al*., 2014; Nguyen and Dang, 2017).

Carotenoids, in addition to their dyeing properties, have antioxidant activity (Zanifi *et al*., 2010; Raddatz-Mota *et al*., 2016), helping organisms to counteract oxidative stress. This antioxidant properties also helps to maintain desirable characteristics in food products, preventing oxidation of its components and improving the antioxidant activity of the products where are applied (Falowo *et al*., 2014; López-Hernández *et al*., 2018). Similarly, phenolic compounds are a group of substances whose functional relevance, including the antioxidant activity, has been tested by numerous working groups (Fraga *et al*., 2010; Carocho and Ferreira, 2013; Ginter *et al*., 2014). Achiote seeds contain significant amounts of these compounds, including ellagic acid and salicylic acid (Ciro *et al*., 2014; Vilar *et al*., 2014).

There are several in vitro methodologies to test the antioxidant activity of diverse compounds and extracts. Among them, stable radical methods such as DPPH and ABTS are the most widely used due to their simplicity, reproducibility and versatility. Such methods are considered to follow an electron and/or proton transference mechanism. In other methods such as CUPRAC (Copper reduction), only electron transference due to the reducing power of antioxidant compounds, is involved (Apak *et al*., 2016). These kinds of methodologies to test antioxidant activity must be considered as part of the characterization of vegetal resources, but keeping in mind their scope and limitations.

The aim of this work was to study the effect of bath sonication treatment on the extraction yield of bixin, norbixin, phenolic compounds and on the antioxidant activity of different achiote accessions from the Yucatan Peninsula as part of the work carried out by the achiote Thematic Network of Plant Genetic Resources (REMEFI for its Spanish acronym) to characterize accessions of southeastern Mexico. The importance of the characterization of different achiote accessions available in this region lies in their possible commercial use, as well as in the fact that their value is directly related to the content of bixin present in the seed and this content varies significantly depending on the variety or accession (Scotter, 2009; Dequigiovani *et al*., 2017).

Table 1. Collection sites information of the achiote seed accession studied in this work.

Accession	Region	Collection site	Coordinate	Voucher specimen ID*
E	Campeche, Mexico	Hopelchén	19°34´20.50´´ 89°36´15.94´´	LP10CAM02
NE	Campeche, Mexico	Hecelchakán	20°10´13.06 ´´ 90°0´37.7´´	LP10CAM08
2N	Quintana Roo, Mexico	Chunhuhub	19°35´27.33´´ 88°35´39.03´´	JM10QR03
NAT 02	Yucatan, Mexico	Espita	20°59´55.29´´ 88°18´14.1´´	PC10YUC15
YUC	Yucatan, Mexico	Hunukú	20°50´59.54´´ 88°5´33.8´´	PC10YUC02

*ID corresponding to the germplasm bank of the Chapingo University, Mexico State, Mexico.

2 Materials and methods

2.1 Plant material

The achiote seeds (Table 1) were collected in the Yucatan Peninsula (Campeche, Quintana Roo and Yucatan states) as part of the work done by the achiote network REMEFI for the characterization of different Mexican accessions. The materials were propagated in the Technological Institute of Conkal, located in the state of Yucatan, Mexico, and voucher specimens were deposited in the germplasm bank of the Chapingo University, in Mexico State. Following collection, samples were sun-dried and stored in low light and low humidity conditions.

2.2 Obtaining of hydrophilic and hydrophobic extracts

2.2.1 Reference method: agitation

The methodology reported by Mène-Saffranè and col. (2010) was used with slight modifications. Briefly, 0.5 g of seed were homogenized in a high-speed homogenizer GLH-01 (Omni International, USA) with 25 mL of 96% v/v ethanol in centrifuge tubes, followed by the addition of 30 mL of dichloromethane (DCM). The mixture was vortexed for 5 min. Then, 50 mL of water was added, vortexed for 5 min and centrifuged at 2000 g for 10 min at 4°C. The aqueous and organic phases were collected separately. The aqueous phase (hydrophilic phase) was gauged to 100 mL and stored at -70 ° C. The plant material was reextracted twice with new addition of water: DCM (30:30 v/v). The new aqueous phases were discarded and the organic phases were mixed and gauged to 100 mL (organic phases). Samples were stored at -70°C until analysis.

2.2.2 Bath sonication method

0.5 g of seed were homogenized in 25 mL of 96% v/v ethanol. Subsequently, 90 mL of DCM were added. The samples were subjected to bath sonication (Branson model 3510, 100 W, 42 kHz, USA) for 20 min reaching a temperature of 45°C. After sonication time, 50 mL of water were added to the mixture and vortexed for 30 s. The sonication conditions (time 10-30 min, power of sonication equipment 80-100 W) and temperature of extraction (ice bath or room temperature 22±2 ºC), were previously selected to obtain the best results in a previous more detailed study (factorial experiments analyzed by ANOVA, data not shown). The extract was centrifuged at 2000 g for 10 min at 4°C and the aqueous (hydrophilic extract) and organic (hydrophobic extract) phases were collected separately. Each phase was gauged to 100 mL with water or DCM and stored at -70 ° C until analysis.

2.3 Norbixin extraction

2.3.1 Reference method: agitation

The FAO (2006) and NOM-119-SSA1-1994 methodology was used. 5 mL of 0.1 N NaOH were added to 0.5 g of whole seeds and vortexed for 5 min. Samples were left standing for 10 min and the supernatant was collected. The plant material was reextracted with another 5 mL of 0.1 N NaOH,

vortexed again for 5 min and allowed to stand for 10 min. The supernatant was recovered and the seeds were discarded. Both extracts were mixed and gauged to 10 mL with 0.1 N NaOH. Norbixin was determined immediately.

2.3.2 Bath sonication method

The extraction with bath sonication was conducted following the method reported by Yolmeh and col. (2014) with the following modifications. 10 mL of 0.1 N NaOH were added to 0.5 g of whole seeds, and sonicated in the bath ultrasonic equipment for 20 min. Final temperature was 50°C. The extracts were allowed to stand 10 min before collecting the supernatant and were gauged to 10 mL. These sonication conditions were previously selected to obtain the best results in a previous more detailed study.

2.3.3 Determination of bixin and norbixin by HPLC

Bixin and norbixin were determined by HPLC according to the method reported by Rahmalia and col. (2015). The analysis of extracts was performed in a Waters 600 equipment (Waters Corporation, USA) equipped with a PDA (Waters 2996) and autosampler (Waters 717plus). The detector was used in a range of 350 to 550 nm in order to obtain the absorption spectra and maximum wavelength. An Econosphere C-18 column (250 x 4.6 mm, 5μ; Grace, USA)) was used and the mobile phase consisted in acetonitrile: 0.5% acetic acid (70:30 v/v) in isocratic run at 1.6 mL/min. Calibration curves of bixin and norbixin were generated and areas at 460 nm were used for the quantification of these compounds.

2.4 Total phenolic compounds determination

Phenolic compounds were determined according to the method of Singleton and col. (1999). 200 μL of sample were mixed with 1 mL of the Folin-Ciocalteu reagent (0.1X) and incubated 1 min before adding 0.8 mL of 7.5% w/v sodium carbonate. It was stirred 10 s and allowed to react for 1 hour in darkness at room temperature. The spectrophotometer absorbance was measured at 765 nm. For the quantification, a standard curve of gallic acid was performed and results were expressed as mg EAG/gdw.

2.5 Antioxidant activity determination

2.5.1 ABTS radical method

The methodology reported by Re *et al*. (1999) was followed. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical was generated by allowing the ABTS solution to react with potassium persulfate in distilled water for 16 h. Subsequently, the ABTS. solution was diluted in 1X phosphate buffer saline solution (PBS) (138 mM NaCl, 3 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4) for hydrophilic fractions or in 96% v/v ethanol for hydrophobic fractions up to an absorbance of 0.7 at 734 nm. 100 μL of the extract were mixed with 1000 μL of the ABTS. diluted solution. The mixture was allowed to stand in darkness for 15 min and the absorbance at 734 nm was measured. Quantification was performed using a Trolox standard curve (one curve for each dilution medium of ABTS). The results were expressed in μmol eq. Trolox/gdw.

2.5.2 DPPH radical method

It was performed following the method reported by Brand-Williams and col. (1995). A 0.5 mM DPPH. (2,2-diphenyl-1-picrylhydrazyl) solution was prepared and diluted in methanol until obtaining an absorbance of 0.9 measured at 515 nm. 50 μL of extract were added to 950 μL of diluted DPPH. solution. The mixture was incubated at room temperature in darkness for 30 min and subsequently, absorbance was measured at 515 nm. Quantification was performed using a Trolox standard curve, results were expressed in μmol eq. Trolox/gdw.

2.5.3 Copper reduction (CUPRAC) method

The method reported by Büyüktuncel and col. (2014) was used with the following modifications: 1 mL of 10 mM Cu^{II}, 7.5 mM neocuprine, 1 M ammonium acetate buffer pH 7 and 0.6 mL of water were mixed. Then, 0.5 mL of hydrophilic sample were added. After 30 min the absorbance of the mixture was measured at 450 nm. For hydrophobic samples, a concentrated solution of Cu^{II} (100 mM) was prepared in water and diluted to 10 mM in 96% ethanol. In addition, instead of adding water, 0.6 mL of 96% v/v ethanol were added. These changes were made to facilitate the dissolution of hydrophobic samples, which were dissolved in DCM. Quantification was performed using a Trolox standard curve; two curves were made, one for the aqueous reaction medium and one for the ethanolic

reaction medium. Results were expressed in μmol eq. Trolox/gdw.

3 Results and discussion

3.1 Bixin and norbixin quantification in achiote extracts

By one hand, the bixin contents in the hydrophobic extracts obtained by agitation were similar among the studied achiote accessions (16.30-20.67 mg/gdw), except for the YUC accession, which presented the lowest values (14.83 mg/gdw) (Figure 1A). These contents are within the range reported for other Yucatan Peninsula achiote accession (8-32 mg/gdw; Raddatz-Mota *et al*., 2016). Broadly speaking, contents from 10 mg/gdw to 70 mg/gdw have been reported in accessions from different regions of the world (Cardarelli *et al*., 2008; Chisté *et al*., 2011; Dequigiovani *et al*., 2017). DCM was used for these extractions, since it is the second most suitable solvent to extract bixin and norbixin from achiote seeds, after chloroform (Giridhar *et al*., 2014). The contents of norbixin in these extracts were between 2.44-3.96 mg/gdw, with YUC accession having the lowest norbixin content (2.24 mg/gdw; Figure 1B). These values are higher than those reported by Raddatz-Mota and col. (2016). On the other hand, when extractions were made in alkaline medium, norbixin was found to be the main pigment in the extracts, followed by bixin (Figure 1C and 1D).

This result could be related to its chemical structure. The presence of two carboxyl groups makes the norbixin molecule prone to undergo the conversion of acidic groups to ionic groups, allowing its solvation by water molecules in aqueous alkaline solutions. Bixin is more resistant to these phenomena, due to the presence of a resistant methoxyl substituent. Therefore, heating bixin-alkaline solutions leads to the conversion of bixin to ionic norbixin (Smith, 2006; Albuquerque and Meireles, 2011). This phenomenon occurred during alkaline extraction and was improved by sonication due to cavitation (Tiwari, 2015; Mercado-Mercado *et al*., 2018). Sonication has been successfully applied to obtain interesting compounds from plant matrices. It has the advantage of being simple, economical, environmentally-friendly not requiring heat treatments, while being able to be combined with a wide variety of solvents (Morales-de la Peña *et al*., 2018).

With this treatment, the bixin contents in the hydrophobic extracts were similar to those obtained by agitation (Figure 1A). Possibly, both methods were able to extract the highest amount of removable bixin. In contrast, norbixin contents (Figure 1B) were significantly increased by sonication. The conversion of bixin to norbixin in the extracts obtained was in a range of 70-85%.These results were confirmed by HPLC analysis. In Figure 2 is observed that hydrophobic extracts had a high bixin (retention time 6.30 min) signal and a low norbixin (retention time 3.48 min) signal, but when extraction was done with 0.1 N NaOH, relative signals of bixin and norbixin were inverted, this conversion being promoted by sonication. Despite of this, the absorption spectra of the whole extracts were not altered by the extraction medium or by the sonication treatment, being the same absorption spectra of bixin and norbixin carotenoids (Figure 2C). These spectra have the reported characteristic form for the bixin and norbixin absorption spectra (Rahmalia *et al*., 2014; Gomez-da Silva *et al*., 2018). The presence of bixin-derivates due to transesterification reaction (conducted by the presence of ethanol) was only detected in alkaline extracts when these were diluted in EtOH, but not when were diluted in acetonitrile or in hydrophilic extracts.

These results are relevant for obtaining norbixin from achiote seeds in a single operation using a more powerful ultrasonic equipment; currently, norbixin is obtained in a two steps method which consist in bixin extraction and its conversion to norbixin by heating in alkaline solutions (Smith, 2006; Scotter, 2009; EFSA, 2016).

3.2 Total phenolic compound yield in achiote extracts

Phenolic compounds have a wide variety of biological activities and are currently under intense study (Fraga *et al*., 2010; Li *et al*., 2014; Izuchukwu *et al*., 2015). They contribute to an important part of the antioxidant activity present in polar extracts from various vegetable sources (Carocho and Ferreira, 2013; Oroian and Escriche, 2015). The phenolic contents in extracts of accessions studied in this work (2.088-2.610 mg/gdw) were within the previously reported ranges (Cardarelli *et al*., 2008; Giorgi *et al*., 2013; Raddatz-Mota *et al*., 2016), with the higher phenolic contents observed in the accessions NE and YUC.

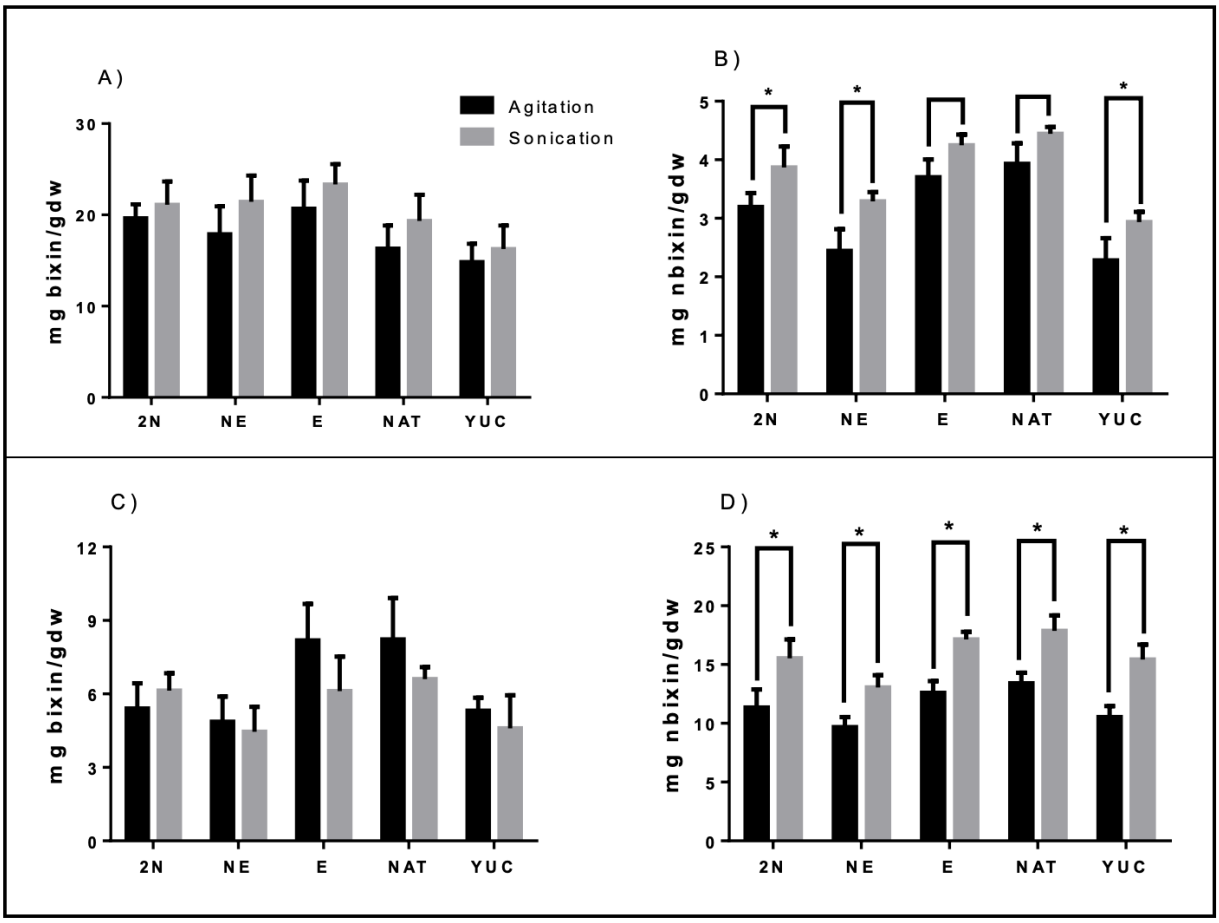

Fig. 1. A) and C) bixin content and B) and D) norbixin content in hydrophilic (upper) and alkaline (lower) extracts obtained by agitation and sonication.

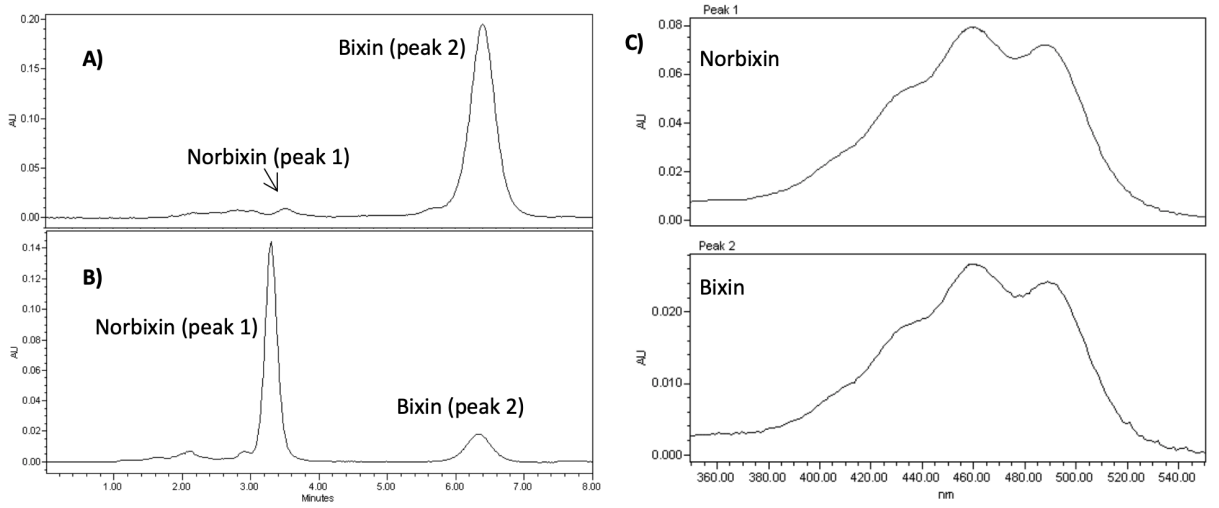

Fig. 2. HPLC chromatograms of A) hydrophobic extracts and B) alkaline extracts of the achiote accession E. C) Absorption spectra between 350-550 nm of the two main compounds, norbixin and bixin.

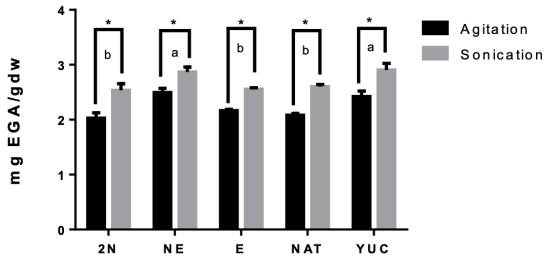

Fig. 3. Content of total phenolic compounds in achiote seed extracts of different accessions obtained by agitation and by sonication. Different letters indicate significant difference between accessions, asterisks indicate difference between treatments according to the Tukey test ($\alpha = 0.05$).

The content of these compounds in the achiote seed extracts was significantly increased by sonication treatment compared to the agitation method (18-25%; Figure 3). Quiroz-Reyes and col. (2013) reported the use of bath sonication to increase the extraction of polyphenols from cocoa beans. These authors also reported the increase in the antioxidant activity of the extracts in comparison with extracts obtained by maceration.

3.3 Antioxidant activity in achiote extracts

In vitro antioxidant activity was evaluated by three different methodologies, the DPPH and ABTS radical methods as well as the copper reduction (CUPRAC) method. While it is accepted that different methods provide complementary information and the use of more than one of them is recommended when a sample is studied, not all methods are applicable to all type of samples (Huang *et al*., 2005).

Figure 4A shows the antioxidant activity measured by the three methods in hydrophilic extracts obtained by agitation. Before separation of hydrophilic and hydrophobic phases, the upper phase is transparent and the lower one is colored. The hydrophilic phase does not contain carotenoids and its antioxidant activity is given by compounds of polar nature, such as polyphenols (Vilar *et al*., 2014; Raddatz-Mota *et al*., 2016). The results of antioxidant activity measured by the three methods showed similar behavior among the different accessions, with higher values for the ABTS method. Similar behavior has been reported for other plant extracts when these methods of antioxidant activity measurement were applied (Shahidi and Zhong, 2015; Apak *et al*., 2016).

Figure 4B shows the results of the hydrophobic fractions, wherein carotenoids are concentrated. In this case, the antioxidant activity determined by each method differs significantly, being underestimated for the DPPH method and overestimated for the CUPRAC method; both effects are due to the increase in reaction mixture absorbance caused by the presence of carotenoids.

Spectrophotometric methods use absorbance measurement; samples that presents absorbance interferences in the wavelengths of analysis will not be appropriate for such methods and the results obtained will be not reliable (Müller *et al*., 2011). In this sense, DPPH and CUPRAC methods were incompatible with the hydrophobic achiote seed extracts due to the presence of carotenoid pigments, which absorb at the wavelengths of determination of the methods (515 and 470 nm, respectively).

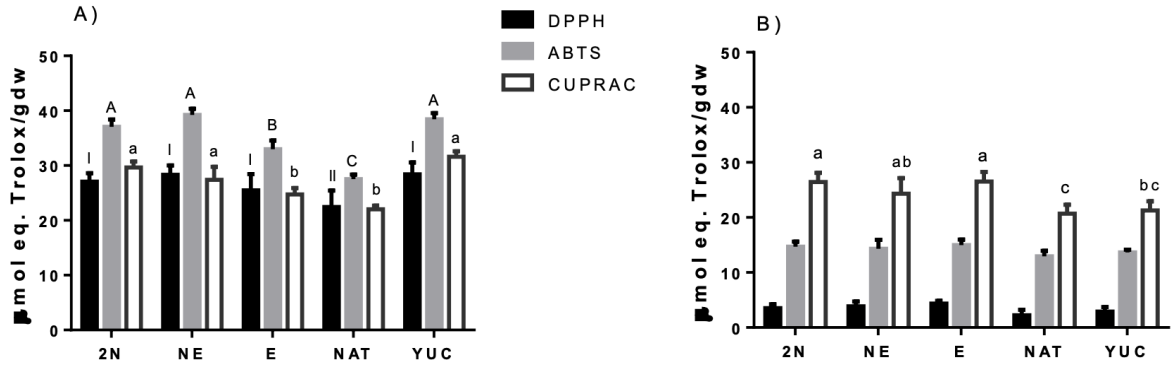

Fig. 4. Evaluation of antioxidant activity by the DPPH, ABTS and CUPRAC methods of (A) hydrophilic and (B) hydrophobic extracts of achiote seeds obtained by agitation. Roman numerals, uppercase letters and lowercase letters indicate statistical differences between accession in each method tested according to the Tukey test ($\alpha = 0.05$).

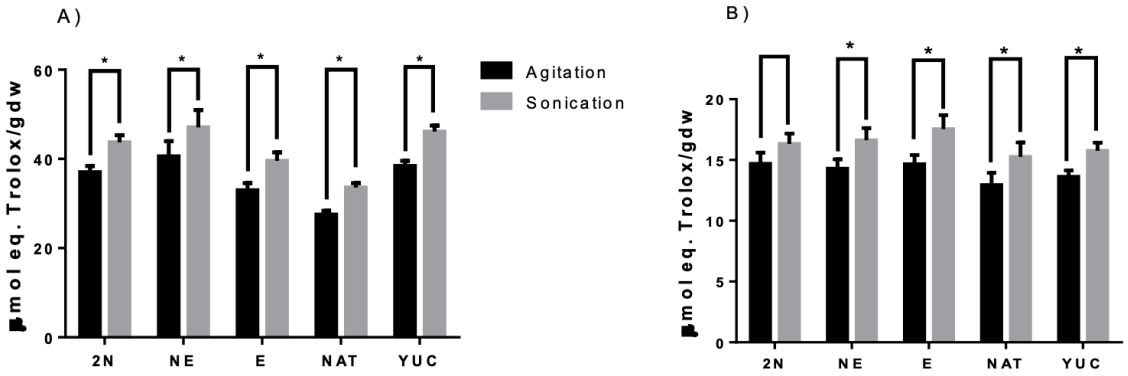

Fig. 5. Determination of the antioxidant activity by the ABTS method of extracts obtained by agitation and bath sonication. A) Hydrophilic fractions, B) Hydrophobic fractions. Asterisks indicate statistical difference between treatments according to the Tukey test ($\alpha = 0.05$).

In the ABTS method, absorbance is measured at 734 nm and no interference occurs. A similar behavior was reported by Müller *et al.* (2011), who found that the ABTS method was adequate to measure the antioxidant activity of many carotenoids, among which was bixin, but not the DPPH method, which presented problems as null reaction, increased rather than decreased absorbance, or lack of a linear relationship between the antioxidant concentration and the antioxidant activity. Due to this, ABTS method was used in the following assays.

The antioxidant activity of the hydrophilic fractions increased with the application of sonication for the five accessions (16-22%); this increase was in line with the augmentation of phenolic compounds with this method. Accession NE had the highest values and accession NAT had the lowest (Figure 5A). In the hydrophobic fractions, accession E had the maximum antioxidant activity, while accession NAT had the lowest. Similar to hydrophobic fractions, the antioxidant activity was also increased with sonication method, in comparison to agitation (12-20%; Figure 5B). Interestingly, phenolic compounds values correlated with the hydrophilic antioxidant activity (agitation P=0.010, sonication P=0.020) and bixin contents but not norbixin contents correlated with the hydrophobic antioxidant activity (agitation P= 0.044, sonication P=0.045).

Although the achiote seeds are appreciated mainly due to their hydrophobic components, the measures realized in this work reflect a greater antioxidant activity of the hydrophilic fractions (12.93-17.54 vs 27.53-47.05 μmol eq. Trolox/gdw). This behavior has been observed in other reports for achiote (Cardarelli *et al*., 2008; Raddatz-Mota *et al*., 2016) and for other fruits with an important content of carotenoids and vitamin E, such as tomatoes (Toor and Savage, 2005; Ilahy *et al*., 2011). However, in vitro results should be complemented in future studies in biological assays (Shahidi and Zhong, 2015).

Conclusions

Achiote accessions 2N and E have potential commercial applications, since they reach a level of 2% of bixin, which is the minimum desirable content. As expected, bixin was the most abundant carotenoid in hydrophobic extracts in relation to norbixin, although this relation was inverted in alkaline extracts. In relation to the content of polyphenolic compounds, NE and YUC accessions presented significantly higher contents than the rest of the accessions. Sonication is a versatile environmentally-friendly tool that increases the extraction efficiency, reducing the time of extraction by 33%. The application of bath sonication allowed to improve the extraction yield of polyphenolic compounds, which resulted in an increase of the antioxidant activity of hydrophilic extracts. The antioxidant activity of the hydrophobic extract was also increased. Since bixin content in sonicated extracts was not significantly increased, the enhancement of antioxidant activity was probably due to the increase of other hydrophobic antioxidants present in achiote seed, such as Vitamin E. The antioxidant activity measured by the ABTS radical was considered to be more reliable for the achiote

hydrophobic extracts obtained. The applied sonication did not help to increase the bixin content in the extracts, but it increased the content of norbixin until 85%. This was possibly due to the participation of ultrasound as a catalyst for the ionization reaction that occurs between bixin and alkaline solutions. This suggests that the application of sonication, maybe with more powerful equipment than that used in this work or with a probe sonication equipment, allows extract norbixin with higher yields.

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Nomenclature

DCM	dicloromethane
HPLC	High Performance Liquid Chromatography
ABTS	2,2'- Azinobis (3-ethylbenzothiazoline-6-sulfonate)
DPPH	2,2-diphenyl-1-picrylhydrazyl
CUPRAC	Cupric Reducing Antioxidant Capacity
gdw	gram dry weight
GAE	gallic acid equivalents

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