International Journal of Phytocosmetics and Natural Ingredients 2014;1:3

doi 10.15171/ijpni.2014.03

Original Article

Antioxidant activity and physicochemical characterization of raw and encapsulated annatto

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Received 13 Jan. 2014
Accepted 24 Mar. 2014
ePublished 10 Jun. 2014

Abstract

Bixa orellana species is originated in tropical America and is widely used in the industry of natural colorants. The annatto colorant has a reddish coloration due to the presence of the carotenoid bixin in its seed aril. The objective of the present work was evaluating the antioxidant potential and characterize the physicochemical properties of three annatto cultivars in fresh (seeds) and encapsulated powder. Therefore, moisture, ash, lipid, protein and carbohydrates were analyzed. To evaluate the antioxidant properties the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) method was used. The annatto seeds and encapsulated products showed antioxidant potential for free radical ABTS, being superior to the synthetic antioxidant BHT which showed 63.62% of inhibition. These results suggest that the use of annatto colorant can provide a preventive action on diseases contributing in a beneficial way to human health.

Keywords: Natural colorant, Free radical, Bixin, Phenols, *Bixa orellana*

Introduction

The annatto (*Bixa orellana* L.) is a native tree of tropical America, belonging to the botanical family Bixaceae.[1] The fruits are capsules that open into two equal parts coated with flexible spines containing numerous seeds. On the seed aril there is a thin layer of reddish pigment, which has been used by the indigenous as medicinal application, and also as ornament and insect protection, by painting their skin.[1]

The pigment is widely used in cooking and food industry in the form of natural colorant, representing 90% of the colorants used in Brazil, and 70% worldwide.[2] In general, in Brazilian industry the annatto is used to color butter, cheese, sausages, ice creams, soft drinks and bakery products. Textile, pharmaceutical and cosmetics industries the annatto is also used in many products.

The annatto pigment can be marketed in various ways, especially as liquid or pastes extract and powder. Another form of marketing that is becoming popular is microencapsulated, due to high demand for herbal products lately. This technique allows the encapsulated product a higher protection from light and therefore increasing its shelf life and thereby increasing its value.[3]

Annatto has many bioactive compounds, which are derived from the secondary metabolism of plants trees. Among the main compounds of annatto phenolic and carotenoids are involved in the functional properties, including antioxidant activity, which is responsible for protecting the cell oxidation and can act preventively against cancers, cardiovascular disease and aging.

These compounds act as antioxidants in protecting the human body against free radicals. These antioxidant defenses can be naturally produced or consumed through the diet. In addition to the protective effects of endogenous antioxidants, the inclusion of antioxidants in the diet is of great importance and consumption of fruits and vegetables is associated with a decreased risk of developing diseases associated with accumulation of free radicals.[4]

The objectives of the present research were evaluating the antioxidant potential and characterizing the physico-chemical of raw and encapsulated annatto.

Material and Methods

Seeds from three annatto cultivars (Peruana Paulista, Embrapa 37 and Focinho de Rato) were used. It was also analyzed three brands of annatto encapsulated powder, identified as A, B and C.

Annatto seeds from the three cultivars were dried in a closed circulation chamber during 72 hours at 55 °C. Later, they were triturated using a ball mill MA 350 and classified in sieves with particle size equal to 180 microns in order to reach particle size near the annatto powder encapsulated. The fractions obtained were packed in polyethylene bags and kept in dark colored dry place at room temperature (25 ± 1 °C), protected from light.

For physico-chemical analysis triturated seeds and encapsulated powdered annatto samples were used.

Moisture was determined by drying samples in chamber, at temperature of 105 °C ± 3 °C for 24 hours using 5 g of ground samples, according to the Standards

Please cite this paper as: Rebouças TNH, Moreira VS, de Moraes MOB, José ABS, da Silva MV. Antioxidant activity and physicochemical characterization of raw and encapsulated annatto. International Journal of Phytocosmetics and Natural Ingredients 2014;1:3. doi: 10.15171/ijpni.2014.03

Analytical Instituto Adolfo Lutz[5], with values expressed in percentages.

The ash content was determined according to the methodology proposed by AOAC.[6] Two g of ground sample was subjected to incineration in a muffle furnace at 550 °C during 4 hours until obtaining a whitish gray residue color (values expressed in percentages).

Protein content was determined by the Kjeldahl method proposed by Silva and Queiroz[7] collecting the released ammonia from boric acid (4%), with values expressed in percentage, using the conversion factor (6.25) for nitrogen/protein.

Total lipids were determined according to the procedure recommended by AOAC[6], using a Soxhlet apparatus extractor, using 1 g of sample with petroleum ether as solvent extractor during 8 hours (values expressed in percentages).

Total carbohydrate content was determined by difference according to the methodology proposed by Sniffen et al.[8], determined by the expression TC = 100 - (% CP +% EE +% MM), where TC = total carbohydrates, CP = crude protein, EE = ether extract/lipids, MM = mineral matter/ash. Values expressed in percentage.

For the determination of antioxidant activity, ethanol extracts was required, so it was used 1 g sample dehydrated kept in ultrasonic bath in three times of 25-minutes, adding 5 ml of ethyl alcohol (80%) each time in a room temperature (25 ° ± 2 °C). After that, the mixture was centrifuged at 4000 rpm for 30 minutes and the supernatant was transferred to test tubes and stored at -18 °C.

The antioxidant potential was determined using the method of discoloration of ABTS.[9] To perform the assay used a radical cation ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)).

A stock solution of ABTS was diluted with a saline buffered phosphate (pH= 7.2) until the absorbance value of 0.70 ± 0.02, determined at 734 nm.

The capture of free radical was expressed in inhibition percentage of radical cation ABTS, according to the following formule:

$$\%\ Inhibition = \frac{A_{white - A_{test}}}{A_{white}} \times 100$$

where,

A_{white} = absorbance at 734 nm of ABTS solution;

A_{test} = absorbance at 734 nm of ABTS solution, 5 minutes after the addition of 1 ml of the sample solution.

A randomized (CRD) experimental design was adopted, with two groups of three treatments with three replicates. Data were subjected to a variance analysis (ANOVA), and comparisons among media were made through the Student t-test with significance level of 5% using the statistical program (SISVAR 4.2).

Results and Discussion

Physico-chemical characterization

The values found for moisture, ash, lipid, protein and carbohydrate in raw annatto seeds are presented in Table 1.

The percentage of moisture in annatto seeds for Embrapa 37, Focinho de Rato and Peruana Paulista cultivars, ranged between 5.91 ± 0.97 and 7.01 ± 0.06, with no significant difference among them. Those values were not far from those found by Lemos[10], which presented values from 4.34 to 6.06% in seed of Bico de Pato I CPATU 0060 and Peruana Paulista cultivars. Peruana Paulista cultivar presented a moisture of 5.91% in the present work against 5.58% obtained by Lemos.[10]

The crude protein ranged from 11.25 ± 0.19 to 13.23 ± 1.22%, with no significant difference among them. The obtained results are in agreement with those found by other authors. Lemos[10] quantified average values from 10.37 to 12.37%, Pezzato et al.[11] found 11.50% and Pereira et al.[12] found content of 13.53%.

The lipids results ranged from 9.62 ± 1.73 to 13.15 ± 0.49%, with no significant difference among the cultivars. Oliveira and Lemos[10] found values of 17.5% and 18.38%, respectively, higher than the obtained in the present work. Lower values were also found in the literature, Pereira et al.[12] found 2.10%, Carvalho et al.[13] quantified values from 1.97 to 3.98%.

For ash content, the obtained values for the three cultivars were: 4.62 ± 0.23% for Embrapa 37; 4.15 ± 0.12% for Focinho-de-Rato; and 3.99 ± 0.28% for Peruana Paulista. There was no significant difference between Embrapa 37 and Focinho-de-Rato cultivars. However, Embrapa 37 showed a significant difference comparing to Peruana Paulista cv. No difference was observed between Peruana Paulista cv. and Focinho-de-Rato cv. For ash content, Lemos[10] quantified 2.99% and Pereira et al.[12] found 6.62%.

The values of total carbohydrates identified by difference, ranged from 70.98 ± 0.5 to 75.15 ± 1.9%, with no significant difference among them. Theses values are approximated to that one (78,02%) found by Pereira et al.[12] However, the values found by Lemos[10] was around 58 and 57%, respectively. The differences observed among data found in the literature and these ones obtained in this work, probably are due to genetic of cultivars, climate variation and harvest season.

The physicochemical characterization of annatto encapsulated products are presented in Table 2. Values of moisture varied from 9.79 ± 0.95 to 10.70 ± 0.91%, with no significant difference among them. The crude protein

Table 1. Physico-chemical characterization of annatto seeds: moisture, crude protein, lipid, ash and total carbohydrates (values expressed in percentage). UESB, Itapetinga - BA, 2012.

Variables	Embrapa 37	Focinho de Rato	Peruana Paulista
Moisture	6.06 ± 1.24[a]	7.01 ± 0.06[a]	5.91 ± 0.97[a]
Crude protein	11.26 ± 0.27[a]	13.23 ± 1.22[a]	11.25 ±0.19[a]
Lipids	13.15 ± 0.49[a]	10.74 ± 2.91[a]	9.62± 1.73[a]
Ashes	4.62 ± 0.23[a]	4.15 ± 0.12 [ab]	3.99 ±0.28[b]
Total carbohydrates	70.98 ± 0.53[a]	71.89 ± 3.94[a]	75.15 ±1.93[a]

The different letters in the same row differ statistically from each other by Student's t test ($p<0.05$).

values ranged from 5.86 ± 2.73 to 12.11 ± 0.41%; brand B showed significantly higher values compared to other brands. Lipid content were found from 12.07 ± 4.31 to 18.91 ± 5.43%, with no significant difference among them. Ashes percentage varied from 2.19 ± 0.94 to 7.01 ± 3.12, and no significant difference among brands. For total carbohydrate the values ranged from 61.97 ± 8.12 in brand B, to 79.79 ± 3 68% in brand A and 74.37 ± 0.01% in brand C. Brands A and C were significantly higher than the value found in brand B.

Evaluation of antioxidant activity by ABTS

The annatto seeds presents bioactive compounds such as phenolics and carotenoids. Bixin and norbixin are examples of such compounds, which has in its chemical structure a potential antioxidant, which have often been subject of research by different authors.

According to Table 3, the Embrapa 37 cultivar showed 84.99 ± 1.01% of inhibition, while Focinho-de-Rato cv. presented 82.46 ± 1.43% and Peruana Paulista cv. 72.51 ± 1.15%. There were no significant differences among extracts of the three cultivars; the control reached 63.62% of inhibition.

The antioxidant properties of annatto seeds can be confirmed by work that was carried out by Martinez-Thomas et al.[14]; in the referred paper they studied the antioxidant activities with spices, including bixin from *Bixa orellana*, *Origanum vulgare* and saffron (*Crocus sativus)* and also with synthetic antioxidants, and butyl hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate. The authors observed good antioxidant activities in spices and in some tests they were superior to synthetic antioxidants.

In agreement to this results Silva and Takemura[15] verified high antioxidant activity in the annatto samples; also, other tests for antioxidant activity with extracts of *Vermania condensata, Bixa orellana* and *Alternanthera brasiliana* were performed. The species *V. condensata, B. orellana* showed significant antioxidant activities, while *A. brasiliana* extract showed low activity.

Kiokias and Oreopoulou[16] evaluated individually the capacity of annatto extract, lycopene and lutein concerning to promote inhibition of hydroperoxides formation in an aqueous emulsion whose oxidation was stimulated by the use of 2,2'-azobis-amidinopropane (AAPH); it was evidenced the high antioxidant activity of annatto comparing to the other compounds, what is somehow in accordance to the results found in the present study.

In tests of antioxidant activity of annatto genotypes by the method linoleic acid/b-carotene, Lemos[10] met values from 18.91 to 35.26% of inhibition. In the present study, extracts of the considered annatto cultivars exceeded the genotypes mentioned by the author, once in this research it was observed up to 84.99% inhibition to free radicals.

In evaluation of the antioxidant activity by DPPH method with extracts of annatto, beet and Antilles berry, Oliveira Neto et al.[17] found that annatto presented higher antioxidant activity compared to the others extracts. The authors believed that the antioxidant activity of annatto is due to phenolic compounds and also to the presence of carotenoids in the pigment.

The papers found in the literature, corroborate with the results of this research concerning to the high percentage of inhibition to free radicals in *Bixa orellana*.

The percentage of inhibition of ABTS radical by ethanol extracts of annatto encapsulated powder samples showed high values. The encapsulated brand A quantified 86.80 ± 0.93%, brand B 98.41 ± 0.23%, and brand C 94.73 ± 0.12% (Table 3), significant differences were observed among the

Table 2. Physico-chemical characterization of annatto powder encapsulated, brands A, B and C. (amounts expressed in percentage). UESB, Itapetinga - BA, 2012.

Variables	Encapsulated A	Encapsulated B	Encapsulated C
Moisture	10.70 ± 0.91[a]	9.79 ± 0.95[a]	10.51 ± 1.01[a]
Crude protein	5.95 ± 0.13[b]	12.11 ± 0.41[a]	5.86 ± 2.73[b]
Lipids	12.07 ± 4.31[a]	18.91 ± 5.43[a]	17.01 ± 1.01[a]
Ashes	2.19 ± 0.94[a]	7.01 ± 3.12[a]	2.76 ± 0.00[a]
Total carbohydrates	79.79 ± 3.68[a]	61.97 ± 8.12[b]	74.37 ± 0.01[a]

The different letters in the same row differ statistically from each other by Student's t-test ($p<0.05$).

Table 3. Percentage Inhibition of free radical ABTS in ethanol extracts of annatto seeds and annatto encapsulated powder. UESB, Itapetinga - BA, 2012.

Samples	% Inhibition of ABTS	Mark	% Inhibition of ABTS
BHT (test)	63.62 ± 0.11[a]	BHT (test)	63.62 ± 0.11[d]
Embrapa 37	84.99 ± 1.01[a]	A	86.80 ± 0.93[b]
Focinho de Rato	82.46 ± 1.43[a]	B	98.41 ± 0.23[a]
Peruana Paulista	72.51± 1.15[a]	C	94.73 ± 0.12[c]

The different letters in the same row differ statistically from each other by Student's t-test ($p<0.05$).

three brands and control (63.62%).

These results demonstrate that the brand B product is superior to others, as the bioactive compounds present higher values. In addition, this product differs from others by the peculiarity of the capsule is dark material, preventing the oxidation of substances.

No studies concerning to antioxidant activity were found for encapsulated annatto powder. Nevertheless, it was verified that in spite of the seed processing, the inhibition to free radicals in *Bixa orellana* is still high.

Conclusion

Among others physico-chemical characteristics, annatto seeds presents high level of crude protein. The encapsulated annatto powder products present lower bixin content comparing to that on the seed. No differences for phenolic compounds in the seeds are observed for the considered cultivars. Anatto seeds and encapsulated powder products showed antioxidant potential to ABTS free radical. Such results suggest that the use of anatto colorants may provide a preventive action against diseases contributing beneficially to the human health.

Competing interests

The authors declare no competing interests.

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