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Carotenoid composition and antioxidant activity of the raw and boiled fruit mesocarp of six varieties of *Bactris gasipaes*

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SUMMARY. Total carotenoid content and composition of carotenoids of six varieties of *Bactris gasipaes* were determined by spectrophotometry and HPLC, with photodiode array detector. Significant differences in total carotenoid content (1.1 to 22.3 mg/100g) were detected among these varieties. Boiling the fruits for 30 minutes did not affect total carotenoid content, but did change the amount of some specific carotenoids, mainly by the production of *Z*-isomers. Peach palm varieties had the same carotenoids, but in different proportions, presenting mainly, *all E*-β–carotene (26.2% to 47.9%), *Z*-γ-carotene (18.2% to 34.3%) and *Z*-lycopene (10.2% to 26.8%). When antioxidant activity was evaluated using DPPH, it was observed that the variety with higher percentages of β-carotene (54.1%) presented the higher activity. This is one of the first reports in carotenoid content and antioxidant activity in well typified varieties of *Bactris gasipaes*, whose results could have a positive impact in the consumption of certain peach palm varieties.
Key words: *Bactris gasipaes*, Arecaceae, DPPH, antioxidant activity, carotenoids, peach palm

RESUMEN. Composición de carotenoides y actividad antioxidante del mesocarpo crudo y hervido del fruto de seis variedades de *Bactris gasipaes*. Se determinó la concentración y composición de carotenoides a seis variedades de *Bactris gasipaes*, mediante espectrofotometría y HPLC con un detector de arreglo de diodos. Las diferencias en el contenido de carotenoides totales fueron significativas entre las variedades estudiadas (1.1 a 22.3 mg/100g). El hervir los frutos por 30 minutos no afectó de forma significativa el contenido de carotenoides totales, pero sí la concentración de algunos carotenoides, principalmente de los isómeros *Z*. Las distintas variedades presentaron los mismos carotenoides pero en diferente proporción, principalmente: *todo E*-β–caroteno (26.2% - 47.9%), *Z*-γ-caroteno (18.2% a 34.3%) y *Z*-licopeno (10.2% a 26.8%).Al evaluarse la actividad antioxidante con DPPH, se observó que la variedad con el mayor porcentaje de β-caroteno presentó la mayor actividad. Este es uno de los primeros reportes de composición de carotenoides de *Bactris gasipaes* bien tipificados, cuyo resultado puede incidir directamente en el consumo de ciertas poblaciones de Bactris.
Palabras clave: *Bactris gasipaes*, Arecaceae, DPPH, actividad antioxidante, carotenoides, peach palm.

INTRODUCTION

Bactris gasipaes (Arecaceae), also known in Costa Rica as pejibaye, chontaduro in Venezuela, pibá in Panamá, pupunha in Brazil and peach palm in English-speaking countries, has important nutritional value, mainly due to the presence of starch and oils, and high energetic content, similar to the one obtained from corn. This is mostly explained by the presence of unsaturated fatty acids, such as oleic acid, which is its major component, showing higher concentrations than sorghum, corn and soy beans (1-4). Due to the importance of this crop for animal and human feeding purposes, the fruit (mesocarp, skin, and seeds) of *B. gasipaes* has been studied for several purposes: a) to improve the quality of the fermentation process, so animal feeding efficacy can be also improved (5), b) to design and evaluate storage and industrialization processes (6,7), c) to determine the presence of anti-nutritional factors and their potential elimination (8) and d) to be utilized as a substitute for other foods in farm animal diets (9).

In diverse studies it has been reported that the yellow-orange color of the peach palm pulp is due to the presence of carotenoids (1.0 mg/100g – 19.8 mg/100g), being β-carotene, *all-E*-δ-carotene, *E*- and *Z*-γ-carotene the predominant ones (10,11).

Total and individual carotenoid content is very variable among studies, probably due to the fact that different varieties have been assayed, and it is well known that different varieties of a fruit or vegetable can have different carotenoid contents (3,12,13).

Carotenoids, having a structure of conjugated double bonds arranged in a linear array, are powerful antioxidants, which may play a role in protecting the body from numerous diseases that are associated with oxidative stress (14,15). Several studies have shown that there are differences in the antioxidant activity among carotenoids; therefore, it is important to know, not only the amount of total carotenoids, but also the specific carotenoid content for its pro-vitamin A and antioxidant activities (16,17).

Some studies have focused on the role of dietary carotenoids in the prevention of coronary arterial disease (18) and some types of cancer (19). In the case of prostate cancer, dietary carotenoids, such as lycopene, have not been only linked to a lower occurrence of this type of carcinoma, but also have been shown to have some therapeutic potential (20). The preventive effect has been partially associated with their ability to scavenge free radicals (antioxidant activity), which are generated during aerobic metabolism and whose presence is also characteristic of some diseases (15). This is supported by studies that showed an increase of carotenoids in lipoproteins (21) and an increase in plasma antioxidant capacity and lower levels of biomarkers of oxidation stress after consumption of carotenoid rich Regarding the bioavailability of these micronutrients in the cooked fruit, an interesting study using Vitamin A-deficient rats, showed that feeding these animals with diets supplemented with peach palm, increased the levels of Vitamin A in the liver to values compared to supplementing directly with the vitamin (23). Considering that diverse studies have shown that *Bactris gasipaes* has a high carotenoid content, but there are no reports about the differences in the composition of carotenoids among their varieties and given that Costa Rica has an experimental station that grows well typified varieties, we decided to study the carotenoid composition of different varieties, their possible relationship with its antioxidant activity, and also compare the effect of boiling on these parameters. This will allow us to select the varieties with the best nutritional properties to improve the diet in the population and the quality of products made from this crop such as the flour used for pastry making.

MATERIALS AND METHODS

Plant material

Peach palm fruits were collected in the month of September in Los Diamantes Experimental Station, Germplasm Bank of the Universidad de Costa Rica (Guápiles, 10.2 °N, 83.8° W, Costa Rica) with the help of Dr. J. Mora-Urpí and Eng. Carlos Arroyo. The varieties collected were: Darién, Brasil, Bolivia, Guatuso, Colombia and Costa Rica.

All the analyses were carried out in duplicate, both the raw and boiled fruits (30 min in boiled water).

Extraction and determination of total carotenoids

Carotenoids were extracted essentially using the procedures established by Schiedt and Liaaen-Jensen (24). Ten fruits of *B. gasipaes* from each population, both raw and boiled, were peeled and cut into small pieces, and then homogenized in a food processor. The carotenoids of ten grams of the homogenate were extracted several times with acetone until no color was obtained and passed through a mixture of ether: n-hexane (1:1). A sample of this solution was dried out under an atmosphere of nitrogen, dissolved in n-hexane and the absorbance was read at 450 nm. The concentration of total carotenoids was expressed as mg carotenoids/100g of peach palm mesocarp.

Saponification

The lipid carotenoid-rich extract was dissolved in 50 mL of ether and 50 mL of alcoholic 5% KOH solution (aqueous KOH diluted 1:9 with ethanol). The solution was saturated with N_2 and the container was closed and left overnight to allow hydrolysis to occur. The mixture was then transferred to a separation funnel, adding 25 mL of ether:hexane (1:1) and 50 mL of distilled water. KOH was eliminated by successive washings with a solution of 8% NaCl. The organic extract was dried out under a N_2 atmosphere and dissolved in the mobile phase for HPLC.

Separation and quantification of carotenoids by HPLC

The analysis was carried out in an Agilent 1050 HPLC, equipped with diode array detector, quaternary pumps (model HP 1050) and an auto-sampler. UV-visible spectra were obtained between 200 and 600 nm and chromatograms were processed at 450 nm. For all of the samples, carotenoid separation was carried out on two serial C_{18} columns: a 4.6 x 150mm and 5 µm particle size LC18 Supelco and a 4.6 x 250mm and 5 µm particle size Vydac 201TP54, using as mobile phase a mixture of acetonitrile: dichloromethane:methanol (82:13:5 v/v) in a isocratic system. The flow rate was 1.5 mL/min and the column temperature was set at 25°C. Identification of carotenoids was made by comparing pure standards prepared in our laboratory from the pulp of red *Bactris gasipaes* (25-27).

Determination of the antioxidant activity

Antioxidant activity was evaluated measuring the scavenging activity of *B. gasipaes* saponified extracts on 2,2 diphenyl 1-picrylhydrazil (DPPH). Two mL of several extract dilutions (5-50 µg carotenoids/mL) were combined with 1 mL of DPPH (0.25 mM in methanol). The absorbance of the samples and control (2 mL methanol + 1 mL DPPH) were recorded after 2 hr in the dark, at room temperature, at 517 nm. The absorbance was plotted against the extract concentration, and a linear regression was established in order to calculate the IC_{50} (µg of carotenoids/mL), necessary to decrease the absorbance of DPPH by 50 % (28). The value of IC_{50} is inversely proportional to the antioxidant activity.

Statistical analysis

Statistical analysis was undertaken using ANOVA and Tukey´s test. *A p* value <0.05 was accepted as statistically significant.

RESULTS

Total carotenoid content Total carotenoid concentration of six varieties of *B. gasipaes* is presented in Table 1. In each variety, there were no statistically significant differences between the total content of carotenoids in boiled and raw mesocarps. However there were significant differences in carotenoids concentration among the different *B. gasipaes* varieties.

Carotenoid composition

HPLC chromatogram that illustrates the separation of the mesocarp of raw peach palm is presented in Figure 1. After verifying the purity of the carotenoids with the DAD detector, we found in the pulp: *Z*- and *E*-β-carotene, α -carotene, *Z*- and *E*-γ-carotene, *Z*- and *E*-lycopene and xantophylls.

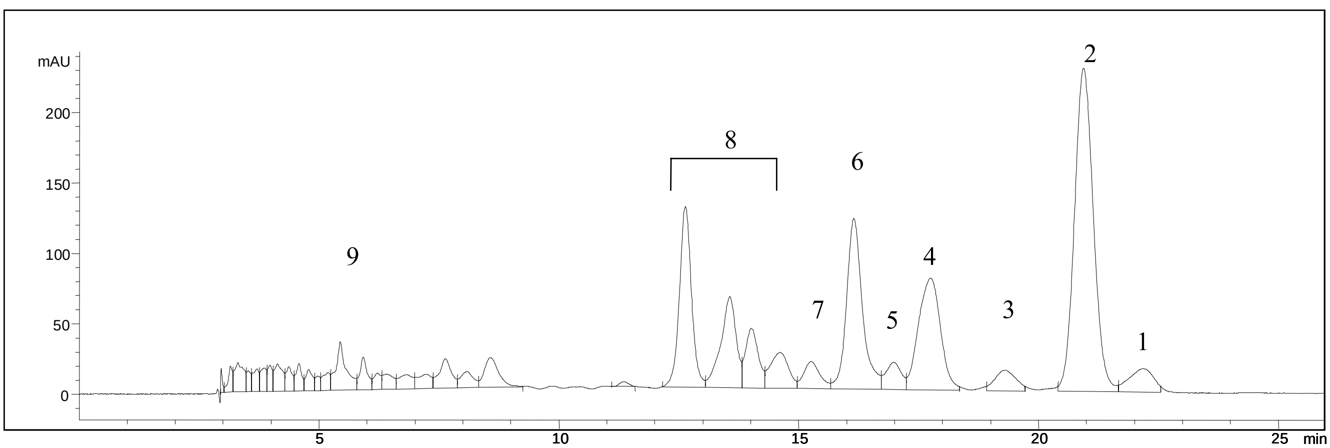
The concentration of specific carotenoids of the raw and boiled mesocarps of six varieties of peach palm is shown in Tables 2 and 3, respectively. In both tables, Bolivia and Darién have the highest amount of all carotenoids; meanwhile Guatuso has the lowest amount of all the carotenoids. In Table 3, it is observed that the proportion of *Z* β-carotene is higher for all the varieties compared with Table 2.

TABLE 1
Total carotenoids obtained from the mesocarp of six varieties of *Bactris gasipaes*

Variety	Fruit color	mg carotenoids/100g of mesocarp raw	boiled
Bolivia	red	22.3 ± 0.6[a]	19.4 ± 1.4[a]
Darién	red	19.3 ± 1.9[a]	21. 1 ± 0.1[a]
Brasil	red	6.4 ± 0.3[b]	6.8 ± 0.23[b]
Costa Rica	red	5.8 ±0.1[b]	5.2 ± 0.01[b]
Colombia	light orange	3.6 ± 0.05[bc]	4.1 ± 0.08[bc]
Guatuso	light yellow	1.1 ± 0.02[c]	1.3 ± 0.07[c]

Each value is presented as mean ± SE. The experiments were performed in duplicate. Means in columns and rows followed by different letters differed significantly ($p < 0.05$).

FIGURE 1
HPLC chromatogram of the saponified extract from the sample of raw mesocarps of the Brasil population.
1. *Z*-β-carotene, 2. E-β-carotene 3. α-carotene, 4. Z-γ-carotene, 5. E-γ-carotene, 6. Z-γ-carotene,
7. *E*-lycopene, 8. *Z*-lycopene, 9. Xantophylls



Radical scavenging activity

DPPH radical scavenging activity of the carotenoid-rich extracts obtained from raw and boiled mesocarps of the six *Bactris gasipaes* varieties is shown in Table 4. All extracts were adjusted to the same concentration of total carotenoids, the scavenging activity was expressed in µg of carotenoids / mL; it was observed that, even though Guatuso had the lowest total carotenoid content, it showed the highest antioxidant activity in both raw and boiling mesocarp.

TABLE 2

Composition of identified carotenoids from raw mesocarp of six *Bactris gasipaes* varieties *(*mg/ 100g of pulp)

Variety	*Z*-β-carotene	*E*-β-carotene	α-carotene	*E*-γ-carotene	*Z* γ-carotene	*E*-lycopene	*Z*-lycopene	Xanthophylls
Bolivia	1.34	8.54	0.7	ND	9.56	0.39	5.22	2.07
	(4.8)	(30.6)	(2.6)		(34.3)	(1.4)	(18.7)	(7.4)
Darién	0.58	5.14	0.50	1.05	5.35	0.59	5.15	0.85
	(3.0)	(26.8)	(2.6)	(5.0)	(27.8)	(3.1)	(26.8)	(4.4)
Brasil	0.19	1.72	0.19	0.14	1.55	0.17	1.60	1.02
	(2.9)	(26.2)	(2.9)	(2.2)	(23.6)	(2.5)	(24.3)	(15.5)
Costa Rica	0.21	1.93	0.29	0.24	2.00	0.14	1.32	0.35
	(3.3)	(29.8)	(4.5)	(3.7)	(30.9)	(2.1)	(20.3)	(5.4)
Colombia	0.34	1.59	0.12	0.08	0.92	ND	0.42	0.62
	(8.3)	(39.0)	(3.1)	(1.9)	(22.5)		(10.2)	(15.2)
Guatuso	0.07	0.51	0.03	0.04	0.19	ND	0.13	0.10
	(6.2)	(47.9)	(2.5)	(3.7)	(18.2)		(12.1)	(9.4)

The value in parentheses corresponds to the percentage of the carotenoid in relation to total carotenoids. ND: < 0.005mg/100g.

TABLE 3

Composition of identified carotenoids from boiled mesocarp of six *Bactris gasipaes* varieties (mg/ 100g of pulp)

Variety	*Z*-β-carotene	*E*-β-carotene	α-carotene	*E*- γ-carotene	*Z*-γ-carotene	*E*-lycopene	*Z*-lycopene	Xanthophils
Bolivia	2.27	4.96	0.68	ND	7.24	0.39	3.97	1.55
	(10.8)	(23.6)	(3.2)		(34.4)	(1.9)	(18.8)	(7.4)
Darién	1.48	4.17	0.74	0.55	6.06	0.84	5.35	0.78
	(7.4)	(20.9)	(3.7)	(2.8)	(30.3)	(4.2)	(26.8)	(3.9)
Brasil	0.63	1.96	0.31	0.21	2.17	0.24	2.20	1.10
	(7.2)	(22.2)	(3.5)	(2.4)	(24.6)	(2.7)	(24.9)	(12.5)
Costa Rica	0.45	1.51	0.34	0.07	1.96	0.16	1.16	0.43
	(7.5)	(24.7)	(5.5)	(1.2)	(32.2)	(2.7)	(19.1)	(7.1)
Colombia	0.64	1.71	0.17	0.05	1.17	0.05	0.48	0.82
	(12.6)	(33.6)	(3.4)	(0.9)	(23.0)	(0.9)	(9.5)	(16.1)
Guatuso	0.13	0.40	0.03	0.03	0.18	ND	0.10	0.08
	(13.5)	(43.1)	(2.7)	(2.7)	(19.2)		(10.7)	(8.0)

The value in parentheses corresponds to the percentage of the carotenoid in relation to total carotenoids. ND: < 0.005mg/100g.

TABLE 4

DPPH radical scavenging activity of carotenoids from raw and boiled mesocarp of six *Bactris gasipaes* varieties

Variety	IC_{50} (µg carotenoids/mL) Raw	Boiled
Bolívia	13.7 ± 0.35^{a}	$24.1 \pm 0.55^{c,\therefore}$
Darién	16.2 ± 0.50^{a}	18.0 ± 0.75^{d}
Brasil	12.5 ± 0.50^{a}	9.5 ± 0.85^{e}
Costa Rica	16.3 ± 1.0^{a}	$10.9 \pm 0.65^{e,\therefore}$
Colombia	12.4 ± 0.50^{a}	$17.4 \pm 0.85^{d,\therefore}$
Guatuso	7.2 ± 0.30^{b}	5.7 ± 0.40^{f}

Each value is presented as mean ± SE. The experiments were performed in duplicate. Means in columns followed by different letters differed significantly ($p < 0.05$); differed significantly ($p < 0.05$) boiled from the raw.

DISCUSSION

The beneficial effects of carotenoids in human disease prevention have been widely reported; they have been investigated for being not only sources of vitamin A, but also for their antioxidant activity (21,29).

Carotenoid values obtained in this study (1.1-22.3 mg/100g) were in the range of those reported by Pacheco de Delahaye and collaborators (3), who determined significant differences in the carotenoid content of twenty ecotypes of *B. gasipaes* of the Venezuelan Amazon, showing values between 3.46 and 40.06 mg/100g. Carotenoid concentrations found in Darién and Bolivia varieties were also similar to the ones reported by deRosso and Mercadante (11) for *B. gasipaes* from the Amazon region.

The higher carotenoid concentration was observed in the fruits with red skin (Bolivia, Darién) and the lowest concentration is observed in the ones with yellow skin (Guatuso, Colombia), showing a correlation between the fruit color and the total carotenoid content. Several studies have previously correlated the color with the carotenoid content of different fruits and vegetables, thus, their color represents a rapid estimation of the amount of this pigment (3,30).

Isomerization of β-carotene from the *E* to *Z* form in all the varieties of *Bactris* after boiling is commonly observed after food processing. *Z*-isomer is commonly found in animal tissues and serum, probably derived from the ingestion of *Z*-isomers of fresh and processed foods (31).

Interestingly, we found substantially higher levels of all carotenoids present in *B gasipaes* than the ones reported by Furtado and collaborators (32). This may be due to the particular population tested or to differences in growing and storage conditions. We used only fresh fruit, due to the fact that in frozen samples, some material could be degraded.

We found that those extracts that had lower carotenoid content, as Colombia and Guatuso, had a higher proportion of β-carotene (47.3 and 54.1%, respectively) while those with higher amounts of total carotenoids, as Bolivia and Darién, had similar proportions of *Z*-γ-carotene and β-carotene.

The composition of β-carotene (*Z*- and *E*-β-carotene) found in the Darién variety was similar to the one reported by deRosso and Mercadante (11). The same was observed with the varieties Brasil and Costa Rica which concentrations of β-, α- and γ-carotene in the boiled pulp were similar to the one reported for *B. gasipaes* from Brazil (29).

According to our results, the varieties of *Bactris gasipaes* with high carotenoid content have a higher β-carotene content than other fruits, such as papaya and mango, which are yellow pulp fruits frequently consumed by the population (29, 33, 34).

Likewise, the Guatuso variety had similar β-carotene content as other fruits, like passion fruit (4.7 µg/g) and guava (5.0 µg/g) (29).

In relation with the antioxidant activity, this study showed that Guatuso had the highest proportion of β-carotene and radical scavenger activity; it is possible that this carotenoid be responsible for the antioxidant activity. When the necessary β-carotene concentration to reach the IC_{50} for each variety was calculated, we found that similar quantities for all varieties (5.2 ± 1.2 µg/mL), were required, which also suggests that β-carotene may be responsible for the detected radical scavenging activity.

However, also it is important to notice that equating the total carotenoid concentration of Guatuso with extracts like Bolivia, which has greater carotenoids content, means that it was necessary to concentrate much more the Guatuso extract, which could lead to also concentrate other lipidic antioxidants, such as tocopherol and tocotrienols that could be present in the pulp of the samples (35).

When comparing the antioxidant activity among the raw and boiled extracts, there was a significant increase in antioxidant activity for the variety Costa Rica, with boiling, and a decrease in the varieties Bolivia and Colombia. Even though it has been shown that heating can induce isomerization of carotenoids, Bohm and collaborators (31), reported that the four β-carotene isomers: *all E*-, *9 Z*-, *13 Z*- and *15 Z*- showed comparable antioxidant activities after heating, therefore the change in antioxidant activity observed should not be based on changes in β-carotene profile. However, this point needs further investigation, because modifications in the profile of other carotenoids present in the extract could play a role in the antioxidant activity.

Higher bioavailability of certain compounds after the boiling of fruits and vegetables has been previously reported by Miglio and collaborators. Such study showed that water-cooking treatments preserve antioxidant compounds, especially carotenoids, and an increase in antioxidant activity is observed in vegetables such as carrots and broccoli, after cooking. They explained the effect based on the release of carotenoids from matrix by heat disruption of protein-carotenoid complexes. It has also been reported that the cooking of vegetables can increase some carotenoids and decrease others, in the way that was observed here, but total carotenoid content could be kept without variations. Yet, more studies are necessary to explain exactly which carotenoids are influencing the antioxidant activity (36).

Peach palm is a fruit with high added value due to the content and quality of its carotenoids. We suggest that this underutilized crop should be more commercialized and this results be use to select the best palms to prepare value added products like jellies, chips or flour for preparation of other different foods.

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