

Carotenoids composition, antioxidant activity and glycemic index of two varieties of *Bactris gasipaes*

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Abstract: Peach palm (*Bactris gasipaes*) is an orange, red or yellow fruit widely cultivated in Central America. It is an energy-rich source of carbohydrates, fats and carotenes, and contains small amounts of protein and fiber. The aim of this study was to describe its carotenoid concentration, antioxidant activity and glycemic index. For the carotenoid composition, antioxidant activity and lipid peroxidation protection two *B. gasipaes* varieties were analyzed: Ecuador and Yurimaguas. Total carotenoids content was 7.43 ± 0.2 mg/100g for Ecuador and 5.74 ± 0.05 mg/100g for Yurimaguas. The amount of each variety necessary to reach 50% radical scavenging activity (DPPH) was 11.6 ± 0.2 mg carotenoids/mL for Yurimaguas and 9.1 ± 0.3 mg carotenoids/mL for Ecuador. In addition, the IC_{50} of the inhibition lipid peroxidation was 11.2 ± 2.1 μ g carotenoids/ml and 10.9 ± 2.2 μ g carotenoids/ml for Yurimaguas and Ecuador respectively. Glycemic index value obtained was 35 ± 6 , which classified *B. gasipaes* as a low GI food. It is concluded that peach palm is an important source of carotenoids and its antioxidant activity and low glycemic index can provide nutritional benefits that may play an important role in the prevention or management of several chronic diseases.

Key words: Peach palm, *Bactris gasipaes*, glycemic index, antioxidant activity

التركيب الكاروتيني ، نشاط مضادات الاكسدة ومؤشر نسبة سكر الدم لصفين من نبات *Bactris gasipaes*

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الملخص إثمار نخلة الخوخ (*Bactris gasipaes*) هي ثمار برتقالية ، حمراء او صفراء اللون ، تزرع بشكل واسع في عدة مناطق من أمريكا الوسطى. تعد هذه الثمار من الثمار ذات المحتوى الغني بمصادر الطاقة لاحتوائها على كربوهيدرات ودهون بالإضافة الى الكاروتينات ، كما تحوي على نسب قليلة من البروتين والالياف. والهدف من هذه الدراسة هو التعرف على محتوى هذه الثمار من الكاروتينات ومضادات الاكسدة بالإضافة الى معرف قيمة مؤشر نسبة السكر في الدم. تم اختيار صنفين من نبات نخلة الخوخ هما Ecuador و Yurimaguas لدراسة محتوى هذه الثمار من الكاروتينات ومضادات الاكسدة وامكانية منع تأكسد الدهون. حسب النتائج التي تم التحصل عليها فقد كان محتوى الكاروتينات الكلية حوالي 7.43 ± 0.2 ملغ/ 100 غرام للصنف Ecuador وحوالي 5.74 ± 0.05 ملغ/ 100 غرام للصنف Yurimaguas. كما اوضحت الدراسة ان الكمية اللازمة من كل صنف لرفع نسبة الفعالية الكاسحة للجذور (DPPH) الى 50% كانت 11.6 ± 0.2 ملغ كاروتين/مل للصنف Yurimaguas و 9.1 ± 0.3 ملغ كاروتين/مل للصنف Ecuador. كما أن النصف تركيز المثبطة القسوى (IC_{50}) لمثبطات تأكسد الدهون قد بلغت 11.2 ± 2.1 ملغ/ 100 غرام كاروتين/مل و 10.9 ± 2.2 ملغ/ 100 غرام كاروتين/مل للصنفين Yurimaguas و Ecuador على التوالي. وحسب نتائج قيمة مؤشر نسبة سكر الدم والتي تم الحصول عليها في هذه الدراسة وهي 35 ± 6 ، يمكن تصنيف ثمار نخلة الخوخ من ضمن الاغذية ذات مؤشر السكر المنخفض. خلصت هذه الدراسة إلى أهمية ثمار نخلة الخوخ كمصدر مهم للكاروتينات ومضادات الاكسدة بالإضافة الى كونها من ضمن الأغذية ذات مؤشر السكر المنخفض مما يزيد من فوائدها من الناحية الغذائية ويزيد من الدور المهم الذي يمكن ان تلعبه للوقاية او السيطرة على العديد من الامراض المزمنة.

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Introduction

Bactris gasipaes has been used for centuries as food by the native population of Central and South America. It is native of rain forest and it grows in a long territory between 16°N and 17°N parallels (Rojas-Vargas et al., 1999; Hernández et al., 2008). It grows very rapidly under the right conditions in regions which altitudes are less than 1000 masl, with abundant but well-distributed rainfall (2000-5000 mm/year), and average temperatures above 24°C (Leterme et al., 2005).

Nowadays it is still cultivated in small scale yielding for two food crops: the fruit and the heart of the palm. The fruit is a drupe with an edible pulp surrounding the single seed, 4-6 cm long and 3-5 cm broad. It grows in bunches of 50-100 units, usually shiny orange, red or yellow when the fruit is ripe, and may have superficial striations (Leterme et al., 2005; Mora-Urpí et al., 1997).

The fruit is frequently stewed in salted water, peeled and dressed with salt and honey, used to make compotes and jellies, or also used to make flour and edible oil. It is considered as a high energy food due to its starch content. The chemical composition is diverse depending on the variety. The mean protein level ranges from 1.8 to 2.7%, lipid levels ranges from 3.5 to 11.1% (Yuyama et al., 2003). It is also known for its content of carotenoids, vitamin C, α -tocopherol, unsaturated fatty acids, like oleic acid and fiber (Leakey, 1999; Mora-Urpí et al., 1997; Clement et al., 2004).

Epidemiological studies have shown a positive correlation between ingestion of vegetables and fruits containing carotenoids and prevention of several chronic diseases such as cancer, inflammation and cardiovascular diseases among others (deRosso and Mercadante, 2007). The health-promoting properties of carotenoids are due to their free radical scavenging activity through the stabilization of single oxygen by its conjugate double bounds (Stahl and Sies, 2005).

Glycemic index (GI) refers to the relative rise in blood glucose occurring after consumption of a food containing a standard amount of carbohydrate (glucose load or white bread) (Monro and Shaw, 2008). Most refined

grain products and starchy vegetables have a high GI, whereas non starchy vegetables, legumes and fruits generally have a low GI. It has been proposed that GI has particular relevance in the management and prevention of chronic diseases such as diabetes, central obesity and insulin resistance among others (Jenkins et al., 2002). Different studies have suggested that GI can be used as a tool to assess potential prevention treatment strategies for those chronic Western diseases associated to overconsumption of refined sugars (Esfahani et al., 2009).

The aim of this study was to determine the carotenoid composition, antioxidant activity and glycemic index of two varieties of *Bactris gasipaes* to consider the nutritional potential of the fruit.

Materials and Methods

Sample Collection

Peach palm fruits were collected at 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 Yurimaguas and Ecuador.

Analytical methods

Carotenoids extraction

Carotenoids extracts were obtained using a procedure previously described (Jatunov et al., 2010). In summary, ten fruits of *B. gasipaes* were peeled and then homogenized in a food processor. The carotenoids of ten grams of the homogenate were extracted several times with acetone 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. The lipid carotenoid-rich extract was saponificated and the organic extract was dried out under an N₂ 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 C₁₈ 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 mesocarp of red *Bactris gasipaes* (Chen and Chen, 1994; Cortés et al., 2004).

Antioxidant capacity

DPPH radical- scavenging activity

The radical-scavenging activity (RSA) of the carotenoids extract was evaluated by assessing their direct DPPH-scavenging activity as described by Jatunov et al. (2010). Briefly, 2 mL of extract dilutions 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 at 517 nm, after 2 hr in the dark, at room temperature. Percentage of radical scavenging activity of the samples was calculated according the formula: % RSA = [1 - (Abs sample/Abs control)] * 100. RSA percentage was plotted against the sample concentration and a linear regression curve was established in order to calculate the IC₅₀, which means that the amount of carotenoids necessary to reach the 50% radical scavenging activity. Results were expressed as µg carotenoids/mL. Each sample was analyzed in triplicate.

Protection to lipid peroxidation in liver homogenates

Sprague-Dawley rats (220g ± 20g) were anesthetized and sacrificed by decapitation according to the Institutional Committee for

Care and Handling of Experimental Animals of Universidad de Costa Rica (CICUA # 19-06). Liver tissue of each rat was obtained and homogenized in phosphate buffered saline (PBS) using an Ultraturrax T-25 equipment (Ika-Labortechnik) to obtain a tissue suspension at 20%. The suspension was centrifuged at 9000rpm during 15 min to reduce suspended solids. Seventy-five microliters of different concentrations of carotenoids extraction were added to 0.75mL of liver-supernatant and incubated for 30min at 37°C. Subsequently, an oxidative stress was induced with TBHP (tert-butyl hydroperoxide) in the final concentration of 1.5mM and incubated for 1h at 37°C. Finally, thiobarbituric acid reactive substances (TBARS) were measured as the end product of lipid peroxidation.

TBARS was assayed according to Uchiyama and Mihara (1978). Briefly, 0.25mL of liver homogenate, 0.25mL of 35% TCA and 0.25mL of Tris-HCl buffer (50mM, pH 7.4) were mixed and incubated 10 min at room temperature. Then, 0.5mL of 0.75% TBA was added and heated at 100°C for 45 min. After cooling, 0.5mL of 70% TCA was added, mixed and centrifuged at 4000rpm for 15 min. The absorbance of the supernatant was measured at 532nm. Concentration of TBARS was assessed using the molar absorption coefficient for malondialdehyde (MDA) of 1.56 x 10⁵cm⁻¹M⁻¹ and results were expressed as nmol MDA/g liver tissue. MDA concentrations were plotted against the sample concentration and a linear regression curve was established to calculate the IC₅₀.

The assay was performed using liver tissue from 5 rats. To establish basal levels, MDA levels without TBPH were assessed in each rat. Due to the color of carotenoids, sample blanks were prepared in each experiment.

Glycemic Index

To determine the Glycemic Index (GI) 12 individuals were selected from both sexes, apparently healthy, nonsmokers, aged between 18 and 51, not overweight, who were not following any diet, had no family history of diabetes or food allergies, and were asked not

to perform strenuous exercise, drink alcohol or eat an unusual amount of food the night before the study. Subjects reported to the test with 12-14 h of fasting, and were given a serving of *Bactris* mesocarp containing 25 g of available carbohydrate. Postprandial blood samples were taken at 30, 60, 90 and 120 min after eating the food according to the protocol of FAO / WHO (1998). They were cited a week after to repeat the test using an amount of white bread containing 25g of available carbohydrates to be used as a standard reference.

This study had the endorsement of the Bioethics Committee of the University of Costa Rica (VI-3300-2008).

Statistical analysis

To compare Yurimaguas and Ecuador results, statistical analysis was undertaken using ANOVA and Tukey's test. A *p* value < 0.05 was accepted as statistically significant.

Results

Carotenoid composition

Table 1 shows the concentration of total and specific carotenoids of the mesocarp of both varieties. There was significant difference in total carotenoids concentration between the varieties. Ecuador variety presents two times the concentration of β -carotene, *Z* γ -carotene and *Z*-lycopene than Yurimaguas variety. HPLC chromatograms of each variety are presented in Figure 1.

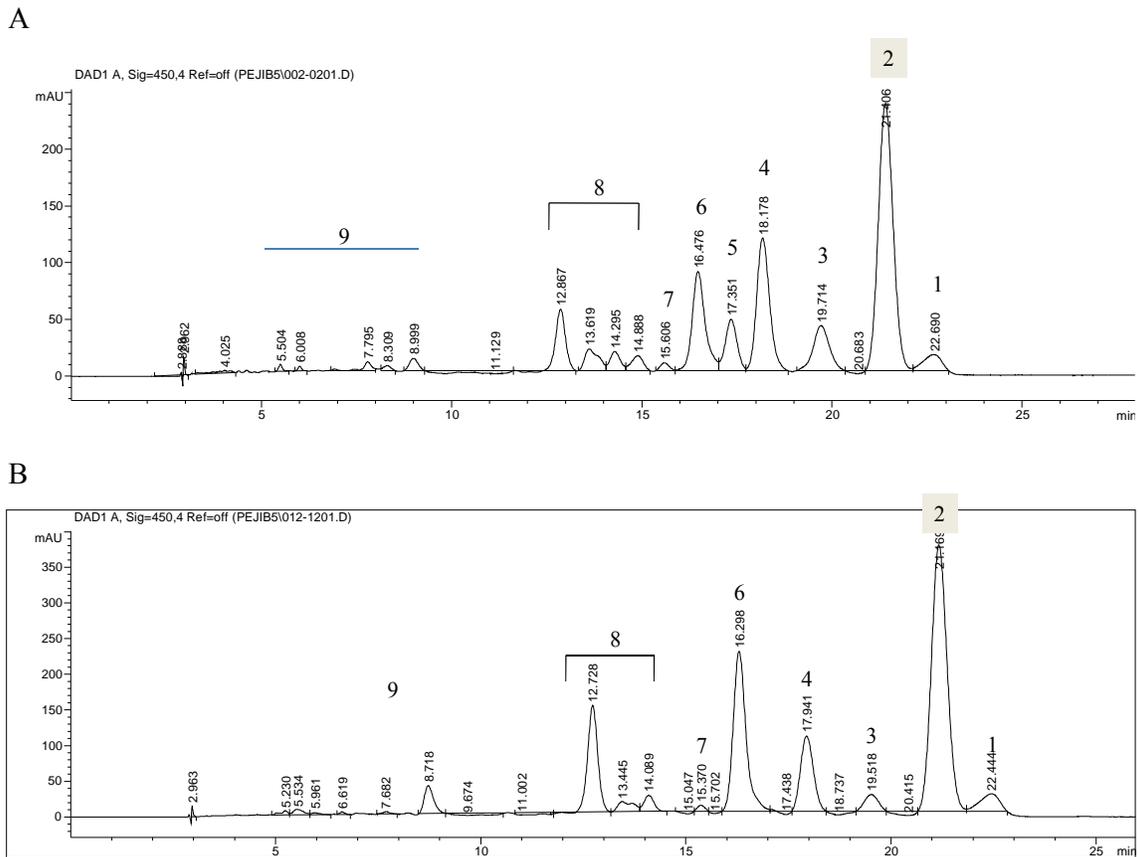


Figure 1. Reversed-phase HPLC separation of carotenoids from the saponificated carotenoids extracts of Yurimaguas (A) and Ecuador (B) varieties. 1. *Z*- β -carotene, 2. *E*- β -carotene, 3. α -carotene, 4. *Z*- γ -carotene, 5. *E*- γ -carotene, 6. *Z*- γ -carotene, 7. *E*-lycopene, 8. *Z*-lycopene, 9. Xanthophylls.

Table 1. Carotenoids composition from mesocarp of *Bactris gasipaes* varieties (mg/ 100g of pulp).

Carotenoid	Ecuador	Yurimaguas
Z-β-carotene	0.31	0.13
E-β-carotene	2.44	1.14
α-carotene	0.26	0.27
E-γ-carotene	ND	0.20
Z γ-carotene	1.95	0.93
E-lycopene	0.08	0.05
Z-lycopene	1.05	0.49
Xanthophylls	0.41	0.24
<i>Total carotenoids^a</i>	<i>7.4±0.2</i>	<i>5.7±0.1</i>

^a Mean ±SE, difference between the varieties was significant, $p < 0.05$. ND: not detected, less than 0.005mg/100g.

Antioxidant capacity

The DPPH assay was utilized to evaluate the antioxidant capacity of both carotenoids extracts (Yurimaguas and Ecuador). The amount of each variety necessary to reach the 50% radical scavenging activity by DPPH method was $11.6 \pm 0.2 \mu\text{g}$ carotenoids/mL for Yurimaguas and $9.1 \pm 0.3 \mu\text{g}$ carotenoids/mL for Ecuador. The difference between the values was significant ($p < 0.05$). BHT was used as a positive control with an IC_{50} of $7.0 \pm 0.2 \mu\text{g/mL}$, showing no significant difference when compared to the Ecuador data.

Protection to lipid peroxidation

To evaluate protection to lipid peroxidation, both carotenoids extracts were tested in liver homogenates of rats induced to oxidative stress with TBHP. Results indicate a hepatoprotective effect against oxidative stress (Figure 2). Yurimaguas and Ecuador carotenoids decrease levels of lipid peroxidation in a dose-dependent manner. The amount of Yurimaguas and Ecuador necessary to decrease 50% of MDA concentration of the control liver tissue treated with TBHP are $11.2 \pm 2.1 \mu\text{g}$ carotenoids/ml and $10.9 \pm 2.2 \mu\text{g}$ carotenoids/ml, respectively. Samples do not show significant differences ($p < 0.05$).

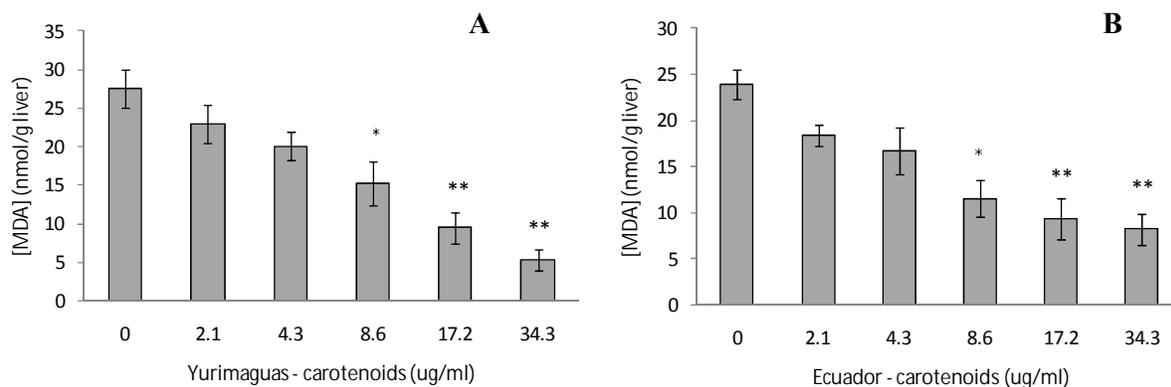


Figure 2. Protective effect of Yurimaguas-carotenoids (A) and Ecuador carotenoids (B) on TBHP induced oxidative stress in liver homogenates model. Each value is mean \pm S.E (five different rats).* $p < 0.05$, ** < 0.01 compared with homogenates treated with TBHP.

Glycemic Index

After the consumption of *Bactris mesocarp*, participants presented the highest glycemia increase in the first 30 minutes of the study,

and reached a minimum value of 80 ± 9 mg/dl from 60 to 90 minutes (Figure 3). The estimated glycemic index of *Bactris*, using white bread as a standard reference, was 35 ± 6 .

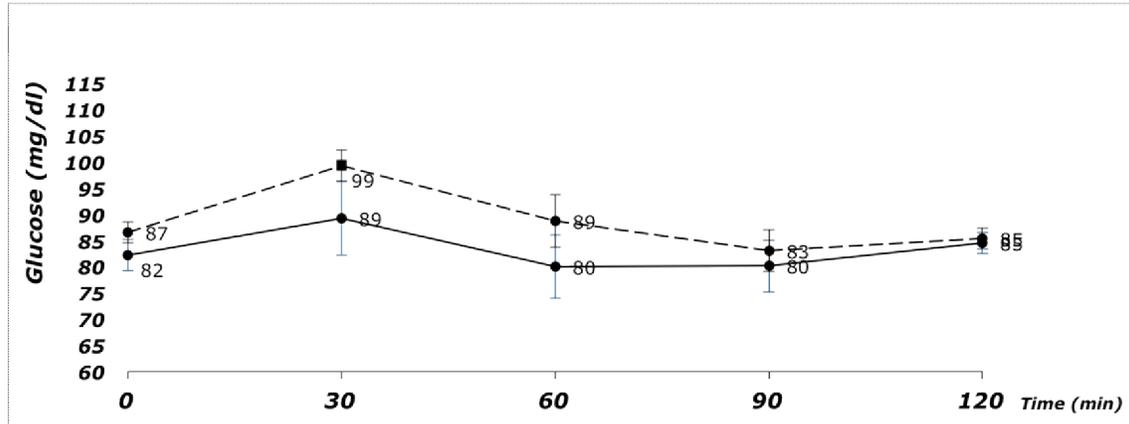


Figure 3. Mean venous glycemic after *Bactris mesocarp* consumption over 120 min test. The values at different points are based on the average blood glucose for each individual \pm SE.
—*Bactris gasipaes* **- -** White bread.

Discussion

Total carotenoids content of both varieties are similar to those reported by Jatunov et al. (2010) of Costa Rica (5.8 ± 0.1 mg/100g) and Brasil (6.4 ± 0.3 mg/100g) varieties. But less than those reported by de Rosso and Mercadante (2007) from Amazonian peach palm (19.7 mg/100g).

Carotenoid content is also greater than other tropical fruits reported by Murillo et al. (2010) like *Citrullus vulgaris* (watermelon, 3.86mg/100g), *Pasiflora edulis* (passion fruit, 3.55mg/100g), *Mangifera indica* (Mango, 3.39mg/100g) and *Carica papaya* (Red papaya, 2.12mg/100g), but similar to those reported by Gil et al. (2002) for nectarine (8-18.6 mg/100g), peaches (7.1-21 mg/100g) and plums (7-26 mg/100g).

Ecuador variety is the one with the greater content of β -carotene, Z γ -carotene and Z -lycopene, and also has a greater free radical scavenging activity. This suggests that those carotenoids could be the responsible for such antioxidant activity, as shown also by Liu et al. (2008). They suggested that the antioxidant property of the combination of β -carotene and

lycopene was substantially superior to the sum of the individual antioxidant effects, and these interactions can enhance the antioxidant effectiveness of natural antioxidants.

Total scavenging activity for Ecuador variety is not significantly different than BHT, making comparable its activity with this antioxidant of commercial use.

Müller et al. (2011) indicated in their study that there was no reaction between the DPPH \bullet radical and carotenoids, nevertheless, we did find scavenging activity through DPPH methodology, maybe due to the extended incubation time (2 hours compared to the 15 min in the procedure used by Müller). Liu et al. (2008) also assessed the radical scavenging activity of carotenoids by the DPPH method.

The *in vitro* protective effects of lipid peroxidation observed in our study on liver homogenates suggest that carotenoids may contribute to enhance antioxidant defense. Upritchard et al. (2003) reported a consistent and significant increase of resistance to oxidation of LDL, after consumption of food products containing moderate amounts of carotenoids and vitamin E. Also Bub et al.

(2000) reported that ingestion of tomato juice reduced LDL oxidation and plasma thiobarbituric acid reactive substances (TBARS) in healthy men.

The Glycemic Index of *Bactris mesocarp* ranks as low; its value is similar to those presented by legumes like red lentils (29), chickpeas (36) and beans (48) and less than some values reported for cereals such as barley (68) and some varieties of rice (64). The glucose contained in low GI food is absorbed more slowly, providing metabolic benefits in diseases like diabetes and the reduction of coronary heart disease risk (Jenkins et al., 2002). The principle is that by slowing the rate of glucose absorption, the insulin demand will be diminished, improving blood glucose control and reducing blood lipid levels (Agustin et al., 2002). Low GI diets have also been reported to improve the decrease of C-reactive protein (CRP) serum concentration, and aid in weight control (Esfahani et al., 2009). In addition, some case-control and cohort studies have found positive associations between dietary GI and risk of various cancers, including those of the colon, breast, and prostate (Esfahani et al., 2009; George et al., 2009).

Conclusions

This work reveals that *Bactris gasipaes* should be considered as a good source of provitamin A carotenes and antioxidant carotenoids and as a low glycemic index food, making it possible to consider its frequent consumption in the prevention of several chronic diseases.

Acknowledgment

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