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Bocaiuva (*Acrocomia aculeata* (Jacq.) Lodd) Improved Vitamin A Status in Rats

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Carotenoid bioavailability is influenced by various factors including the food matrix. The release of those molecules from the food matrix is the initial and most important step in the absorption process. The relative bioavailability of bocaiuva pulp β-carotene in relation to pure β-carotene was assayed by a hepatic retinol store, in vitamin A deficient Wistar rats. After the depletion period, the vitamin A deficient rats were separated into two groups and fed an AIN-93G modified diet, which contained 14 400 μg of pure β-carotene (β-carotene diet) or 13 475 μg of β-carotene from 275 g of bocaiuva pulp (bocaiuva diet) per 1 kg of the diet as a vitamin A source. Both experimental diets resulted in similar body weight gains. The bioavailability, estimated as Retinol Accumulation Factor, was 7.3 and 3.5 for the β-carotene and the Bocaiuva group, respectively. These results show that in spite of the matrix of the bocaiuva pulp, the β-carotene bioavailability from this fruit was higher than the pure β-carotene.

KEYWORDS: Bioavailability; β-carotene; bocaiuva; *Acrocomia aculeata* (Jacq.) Lodd; vitamin A

INTRODUCTION

In spite of its critical role in cell differentiation and proliferation, maintenance of epithelial cell integrity, embryonic development, and in the mechanism of vision in the dark (*1*−*3*), vitamin A deficiency is still a public health problem in the world. It is becoming worse in developing countries, where it coexists with low vitamin intake and other micronutrient deficiencies (*1*). In Brazil, the marginal vitamin A deficiency reaches practically all the regions of the country, even the richest ones (*4*). Vitamin A deficiency affects growth, he differentiation of epithelial tissues, and immune competence. It is the single most frequent cause of blindness among preschool children in developing countries. The younger the child, the more severe the disease is and the higher the risk that corneal destruction will be followed by death (*5*−*7*).

Vitamin A deficiency occurs when body stores are exhausted and the supply fails to meet the body's requirements. The major dietary source of vitamin A in humans consuming predominantly vegetarian diets are the provitamin A carotenoids, which are converted to retinol by the action of 15−15′-carotenoid dioxygenase mainly in the enterocytes from intestine mucosa. This process results in the formation of retinal that could be reduced to retinoid forms, which are incorporated into chylomicrons and secreted into lymph for delivery to the blood stream, where they are taken up by the liver to be stored (*8*, *9*). However, the rate of conversion of these molecules into vitamin A depends on their bioavailability (i.e., their release from the food structure, their processing into a potentially absorbable form, and their passage from gut lumen into the duodenum enterocytes (*10*)). Several factors including the carotenoids in foods, the matrix in which carotenoids are incorporated, and the diet composition influence carotenoid bioavailability (*8*, *10*, *11*). Numerous native Brazilian fruits have been identified as rich sources of carotenoids (*12*). Bocaiuva fruit, *Acrocomia aculeata* (Jacq.) Lodd, is a palm from the cerrado (Brazilian savannas) whose yellow pulp is consumed fresh or processed as ice cream, juice, liqueur, and jelly, with great popular acceptance. A previous report has found that about 89% of the total carotenoid content in the bocaiuva pulp is β-carotene, and the bocaiuva pulp is rich in oils with a high content of unsaturated fatty acid such as oleic acid (*13*, *14*), which may influence the carotenoid bioavailability.

Rats have not been considered to be the most appropriate animal model to investigate carotenoid bioavailability; however, they have been used to evaluate the efficiency of β-carotene conversion to vitamin A by monitoring changes in liver vitamin A stores (*2*, *15*, *16*). Rats are high efficiency converters of

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β-carotene in vitamin A at level of enterocytes; therefore, they do not readily absorb intact carotenoids differently than humans, who absorb small, physiologic doses of a variety of carotenoids, including intact β-carotene (*2*). However, a comparative study of carotenoid bioavailability using rats is very useful to investigate mainly dietary factors such as the food matrix that is one of the first and most critical steps in the carotenoid absorption, once absorption by the small intestine enterocytes occurs via passive diffusion (*8*, *10*), and there is no evidence that this mechanism is different between humans and rats. The aim of this study was to compare the potential benefits of β-carotene from bocaiuva pulp by quantifying the effectiveness of pulp on the recovery of hepatic vitamin A reserves after deficiency induction in relation to pure β-carotene in rats.

MATERIALS AND METHODS

Bocaiuva Pulp. Mature fruits of the *A. aculeata* (Jacq.) Lodd, from the Cerrado Biome (woodland savanna), were harvested between August and November in the city of Campo Grande, Mato Grosso do Sul, Brazil. The ripe stage of the fruits was determined by the fruit firmness and surface color (yellow). Fruits free of defects were selected and manually peeled. The pulp was homogenized with a food processor and stored in plastic bags under vacuum at −70 °C until the analysis.

β-Carotene Analysis. The carotenoids from the bocaiuva pulp fruit (25 g) were extracted and saponified according to the Rodriguez−-Amaya (*17*) and Hiane and Penteado (*13*) methods modified as follows: the ether carotenoid extracts were concentrated in a rotary evaporator and submitted to high performance liquid chromatography (HPLC) analysis, with a photodiode array detector (Waters model 2996) and a Spherisorb OD2 column (4.6 mm × 150 mm i.d.). Acetonitrile (with 0.05% triethylamine)−methanol−ethyl acetate was used as the mobile phase, with a linear gradient 90:10:0 to 60:20:20 for 40 min, at a flow rate of 0.5 mL/min. The β-carotene was detected at 450 nm, and the concentration was determined by the external standard method (*15*). The homogeneous pulp was analyzed in triplicate.

Experimental Design. The design of the study was based on a rat vitamin A liver depletion and repletion method according to Furusko et al. (*15*) modified by Graebner et al. (*19*). Briefly, 20 male Wistar weanling rats 21 days old (45.0 ± 9.5 g) were obtained from the University of Mato Grosso do Sul in Brazil, housed in individual cages in a room maintained at 22 ± 2 °C with a 12 h light/dark cycle, and fed between 4 PM and 8 AM with free access to water. Rats were acclimated during 5 days feeding the AIN-93G (*20*) modified diet, the vitamin A requirement was replaced by β-carotene, and then they were subjected to the AIN-93G diet without any source of vitamin A during 28 days to induce vitamin A deficiency (depletion period). After depletion, four rats were killed to determine basal hepatic retinol. The remaining rats were randomly separated into two groups (eight rats/group), and they were treated, for 21 days (repletion period), with the AIN-93G modified diet, with either 14 400 μg of pure β-carotene powder (Fluka) dissolved in soybean oil (β-carotene diet) or 13 475 μg of β-carotene from 275 g of the fresh bocaiuva pulp (bocaiuva diet) per 1 kg of the total diet, replacing the vitamin A requirements. The diets were stored in dark plastic bags at −20 °C (**Table 1**). At the end of the repletion period, the rats were killed by anesthetic sodium thiopental (thionembutal) in lethal doses, and the liver was excised, washed in ice-cold saline, blotted on paper towels to remove excess blood and saline, weighed, and immediately frozen in liquid N_2 and stored at −70 °C until the analysis. Food intake was monitored daily, and body weight was monitored weekly. The experimental protocol was approved by the Animal Care Committee, Federal University of Mato Grosso do Sul.

Liver Retinol Determination. The liver retinol concentration was measured according to the Tanumihardjo and Penniston (*21*) method, adapted for hepatic retinol. Samples with approximately 0.1 g of liver were homogenized at 4 °C and suspended in ethanol (1.5× vol of the sample) by vortexing for 15 s. To this suspension was added an aqueous solution of KOH 50% (0.8× vol), mixed by a vortex for 15 s and placed into a water bath at 50 °C for 30 min. The sample was mixed every 15 min for about 15 s. After saponification, the sample was extracted 3 times with 2× vol of hexane by mixing for 30 s and centrifuging for 3 min. The top organic layer was pooled into a clean test tube and evaporated under nitrogen gas. The residue was redissolved in 2 mL of ethanol. The 20 μL sample was injected into the HPLC system (Varian, Chromsep 250 mm × 4.6 mm i.d., Onnspher 5 C_{18} column). An internal standard was used during the hepatic retinol extraction procedure to control mechanical and degradative losses. A mixture of methanol/water (95:5) was used as the mobile phase, at a flow rate of 1.5 mL/min. The retinol was detected at 325 nm. The hepatic retinol concentration was determined using a standard curve obtained with an external standard solution of the synthetic all-*trans*-retinol (Sigma-Aldrich). The retinol concentration was calculated by the molar extinction coefficient of 1850 (ethanol) at 325 nm (*22*). Three samples from each liver were analyzed.

Table 1. Composition of Diets According to Formulations of AIN-93G Diet (*14*)[a]

ingredients (g)	DD[b]	CD[b]	BD[b]
cornstarch	397.5	397.5	362.9
starch (*bocaiuva*)			34.6
protein (casein)	200.0	200.0	195.9
protein (*bocaiuva*)			4.1
dextrinized cornstach (90–94% tetras.)	132.0	132.0	132.0
sucrose	100.0	100.0	75.3
sucrose *(bocaiuva*), conversion			24.7
soybean oil	70.0	70.0	47.7
oil (*bocaiuva*)			22.3
fiber	50.0	50.0	12.0
fiber (*bocaiuva*)			38.0
vitamin mix[c]	10.0	10.0	10.0
mineral mix	35.00	35.0	35.0
L-cysteine	3.0	3.0	3.0
choline bitartrate (41.1% choline)	2.5	2.5	2.5
butilhydroxitoluene (mg)	14.0	14.0	14.0
vitamin A source (μg of β-carotene)		14425.5[d]	13475.0[e]

[a] Expressed in g/kg diet. [b] DD = diet without any vitamin A source; CD = β-carotene diet, containing pure β-carotene as vitamin A source; and BD = test diet containing bocaiuva pulp as the vitamin A source. [c] Vitamin mix AIN-93G without vitamin A. [d] Pure β-carotene (Fluka). [e] β-carotene from bocaiuva pulp.

Bioavailability of β-Carotene from Bocaiuva. The β-carotene bioavailability was evaluated as the retinol accumulation factor (RAF) according to Zakaria-Rungakat et al. (*23*). The RAF was calculated by dividing the β-carotene intake (I) by the total retinol accumulation in the liver (LRA). The LRA was calculated by the difference between the total liver retinol found at the end of the repletion period and the total liver retinol found at the end of the depletion period (basal retinol level). The relative bioavailability was determined in relation to the RAF found in the β-carotene group.

Statistical Analysis. Statistical analysis of the data was determined for weight gain, diet consumption, β-carotene intake, hepatic retinol, and relative bioavailability. The results were expressed as the mean value ± standard error of the mean. Analysis of variance (ANOVA) was used to determine differences between diet treatments, with the Excel 2003 (Microsoft Office) program. Differences associated with $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Content of β-Carotene. The major carotenoid identified in the chromatography profile from HPLC of the bocaiuva pulp was β-carotene, representing about 82% of the total peak area of the carotenoid profile. There were also other minor carotenoids not identified in this study (**Figure 1**); however, in earlier studies at our laboratory, these minor carotenoids were identified by an open column chromatography method as γ-carotene, β-cryptoxanthin, *cis*-lycopene, and *cis*-flavoxanthin (*13*). In the present study, the β-carotene content was 49.0 ± 2.0 μg/g (wet weight), similar to the value reported by Hiane and Penteado (59.4 ± 11.1 μg/g) (*13*).

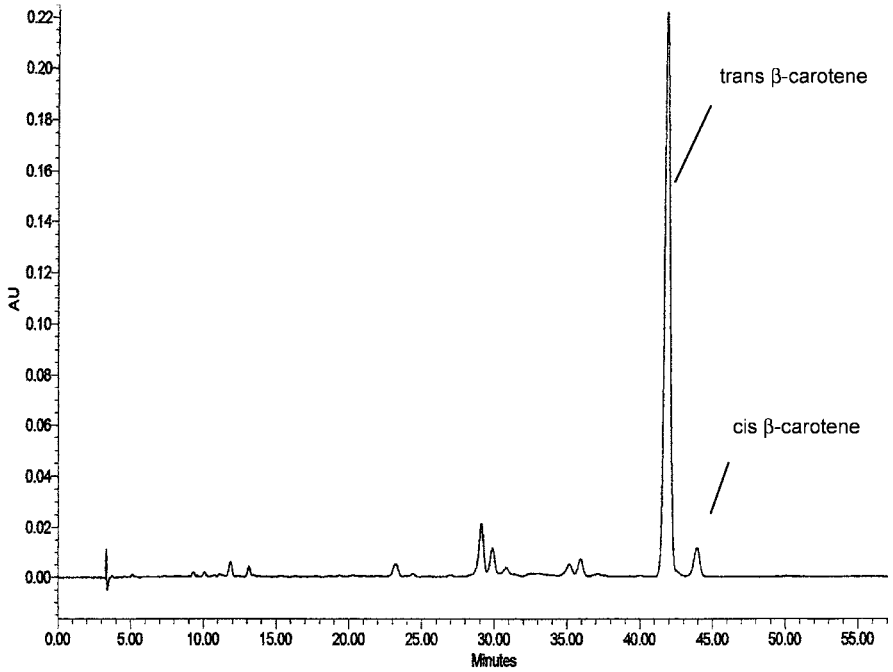

Figure 1. Typical HPLC chromatogram profile of the carotenoids from bocaiuva pulp at 450 nm, obtained through the photodiode array detector. Separation conditions. Column: monomeric Sherisorb OD2 (4.6 mm × 150 mm, 3 μm); mobile phase: acetonitrile (0.05% triethilamine)/methanol/ethyl acetate, in a linear gradient of 90:10:0 to 60:20:20 for 40 min; and flow rate: 0.5 mL/min.

Table 2. Weight Gain, Diet Intake, and Liver Weight of Rats from β-Carotene and Bocaiuva Groups at End of Repletion Period[a]

groups[b] ($n = 8$)	weight gain (g)	diet intake (g)	liver weight (g)
β-carotene	99.3 ± 8.9[a]	323.6 ± 37.3[a]	15.1 ± 2.3[a]
bocaiuva	99.1 ± 21.5[a]	313.4 ± 31.3[a]	14.6 ± 1.7[a]

[a] Means ± standard error; values in columns not sharing a common superscript letter are significantly different ($p < 0.05$) by ANOVA. [b] Rats with vitamin A previously depleted were treated with a modified AIN-93G diet, containing vitamin A replaced by pure β-carotene (β-carotene group) or bocaiuva pulp (bocaiuva group).

Table 3. β-Carotene Intake (I) during Repletion Period, Hepatic Retinol Level (HRL), Liver Retinol Accumulation (LRA), and Retinol Accumulation Factor (RAF) at End of Repletion Period[a]

diet ($n = 8$)	β-carotene intake (I) (μg)	HRL (μg/g)	LRA (μg)	RAF (I/LRA)
β-carotene	5110.5 ± 588.4[a]	47.5 ± 6.5[a]	717.8 ± 141.9[a]	7.3 ± 1.0[a]
bocaiuva	4562.2 ± 456.1[a]	91.4 ± 14.6[b]	1317.1 ± 166.8[b]	3.5 ± 0.5[b]

[a] Means ± standard error; values in columns not sharing a common superscript letter are significantly different ($p < 0.05$) by ANOVA.

The composition of carotenoids in vegetables and fruits is affected by several factors such as cultivar or variety, part of plant consumed, stage of maturity, climate or geographic region of production, harvesting and post-harvest handling, processing and storage (*12*, *23*, *24*), and also the milling processes that result in matrices disruption and delivering of carotenoids from the matrix.

Bioavailability of β-Carotene. Earlier studies have found that rats fed β-carotene replacing vitamin A requirements showed a higher weight gain than the vitamin A deficient rats (*23*, *25*). In the present study, at the end of the depletion period, the rats showed a weight gain average of 150.9 ± 21.3 g and a hepatic retinol concentration of 4.6 ± 1.8 μg/g. After the repletion period, there was no significant difference in the weight gain of the rats from both groups (β-carotene and bocaiuva). There was also no difference in the diet intake and the rats' liver weight between the groups (**Table 2**). These results suggest that β-carotene from bocaiuva pulp may supply enough vitamin A to promote rat growth as pure β-carotene does and also that the calorie and protein value adjustment in the bocaiuva diet was adequate (**Table 1**).

During the repletion period, the rats from the β-carotene and bocaiuva groups increased the liver retinol level from 4.6 μg/g (basal level) to 47.5 and 91.4 μg/g, respectively ($p < 0.0001$) (**Table 3**). These results showed that β-carotene from both diets was able to restore the rat liver retinol. However, in spite of the similar β-carotene intake, during the repletion period, hepatic analysis showed a higher vitamin A accumulation in the rats from the bocaiuva group (**Table 3**).

The β-carotene bioavailability measured by the retinol accumulation factor (RAF) in the rats fed a pure β-carotene diet was 7.3 ± 1.0 and in the rats fed bocaiuva pulp was 3.5 ± 0.5. In other words, these results suggest that 7.3 μg of pure β-carotene produced 1 μg of liver retinol accumulation and that 3.5 μg of β-carotene from bocaiuva produced 1 μg of liver retinol accumulation (**Table 3**). The lowest RAF value reflects the highest bioavailability. Thus, the relative bioavailability of β-carotene from bocaiuva was about 200% of the pure β-carotene bioavailability ($p < 0.0001$).

The direct measurement of liver reserves of vitamin A has been described as the gold standard method to assessing the vitamin A status (*26*); therefore, this indicator has been widely used to evaluate the food effective in improving vitamin A status (*15*, *23*, *27*). Other methods such as isotope ratio and dose−response tests have been validated against liver reserves in either animals or humans (*26*).

Carotenoid bioavailability is influenced by multiple endogenous and exogenous factors such as carotenoid food composition, dietary fat and fiber, matrix properties, food preparation, particle size, and carotenoid interactions during the absorption, metabolism, and transport process (*8*, *28*, *29*, *30*). The first limiting step of carotenoid bioavailability is the release of

carotenoids from the foods that occurs when the plant cell is disrupted during food processing. The second major limiting factor is the carotenoid solubility in the intestinal lumen (*10*). In orange and yellow fruits, carotenoids are dissolved in oil droplets in chromoplasts and can be readily extracted during digestion (*9*), which facilitates the β-carotene delivery into the gastrointestinal tract. Carotenoids appear to be absorbed by small intestine enterocytes via passive diffusion to be packaged into chylomicrons. Release of β-carotene from the matrix and dissolution in the lipid phase are critical steps in the absorption process (*8*, *10*). Food processing, such as mechanical homogenization or heat treatments, results in food matrices disruption that contributes to the increased carotenoid bioavailability (*30*). The improvement of bioavailability of lycopene by mechanical homogenization and heat treatment was confirmed by van Het Hof et al. The bioavailability of lycopene from tomato paste was higher than that from fresh tomatoes; similar effects were found for β-carotene (*31*). Cooked, pureed carrots and spinach seemed to be absorbed approximately 3-fold better than the raw, intact vegetables (*32*).

In this study, the differences between the β-carotene sources, pure or from bocaiuva pulp, both mixed in the rats' diets, were reflected in the liver vitamin A concentration. The β-carotene and bocaiuva groups received diets with similar contents of energy, fat, and other nutrients. Thus, the higher bioavailability of β-carotene from bocaiuva may be due to the form of β-carotene present in the matrix of fruit pulp or in the presence of other molecules such as lipids that may facilitate β-carotene uptake by the enterocyte, after the matrix disruption (*8*, *10*). In previous reports, Yuyama et al. evaluated the bioavailability of carotenoids from other two palm fruits, buriti (*Mauritia flexuosa* L.) and pupunha (*Bactris gasipaes* Kunth), using liver retinol accumulation in rats. The authors also found higher β-carotene bioavailability in the groups fed diets supplemented with buriti and pupunha flours in relation to the retinyl palmitate, the respective control group (*33*, *34*). These results suggest that these Brazilian palm fruits may be a good source of β-carotene and vitamin A for rats; however, the ability of this model to predict bioavailability in a healthy human population needs to be verified.

Several studies have clearly showed that β-carotene bioavailability is strongly influenced by the vitamin A status of the organism (*23*, *35*, *36*). The bioavailability may also be influenced by the time used to replete the animals. An early study, developed in our laboratory, in which depleted rats and liver retinol accumulation were also used to assay β-carotene bioavailability, showed that the retinol accumulation factor was 12, higher than the value obtained in the present study; however, the repletion period was different between the studies (30 and 21 days, respectively) (*19*). The vitamin A status had a large effect on vitamin A and β-carotene storage; thus, a single RAF may not accurately reflect bioavailability under any circumstances (*27*).

New scientific interest in identifying new sources of bioavailable carotenoids has increased in the last two decades not only for their vitamin A value but also because of antioxidant properties of carotenoids. Carotenoids may protect humans and animals against oxidative stress (*37*, *38*). These antioxidant properties have been attributed to the ability of these vegetable pigments to scavenge singlet oxygen and, to a lesser extent, inhibit lipid and protein oxidation reactions (*39*). Previous reports have found a protective effect of carotenoids against several chronic diseases, including cancer and cardiovascular diseases (*3*, *7*, *40*).

In the 1990s, an intervention study showed that dark-green leafy vegetables and carrots had no effect on the serum retinol concentration, which suggested that these foods were a poor source of vitamin A (*41*). However, a recent study, where intrinsically labeled carotenoids were used, showed that spinach and carrots can provide significant amounts of vitamin A in men and women (*42*). In the present study, the provitamin A carotenoids from the native bocaiuva pulp were more effective in improving vitamin A status than the pure β-carotene. These results suggest that bocaiuva fruit may be a very good source of provitamin A carotenoids. This fruit is largely consumed fresh or in a flour form, by the Brazilian savanna (cerrado) population, as candy, ice cream, and other regional plates. Thus, this fruit might contribute to the reduction of hypovitaminosis A incidence and combat the micronutrient deficiency in this region. However, it is always necessary to emphasize that in any animal model of study, including in the present one, the extrapolation of the results for the human organism should be taken with caution and needs confirmation.

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