



Effects of diet supplementation with Camu-camu (*Myrciaria dubia* HBK McVaugh) fruit in a rat model of diet-induced obesity

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ABSTRACT

Amazonian Camu-camu fruit (*Myrciaria dubia* HBK Mc Vaugh) has attracted interest from food and cosmetics industries because of its rich content of vitamin C, flavonoids and anthocyanins. The goal of this study was investigate the antiobesity action of the ingestion of the Camu-camu pulp in a rat model of diet-induced obesity. Wistar rats with obesity induced by subcutaneous injection of monosodium glutamate receiving diet *ad libitum*. The rats were divided in two groups: an experimental group that ingested 25 mL/day of Camu-camu pulp (CCG) and a non treated group (CG). After 12 weeks, the animals were sacrificed. Blood, liver, heart, white adipose tissues were collected and weighted, biochemical and inflammatory profiles were determinate as well. Animals that received the pulp of Camu-camu reduced their weights of the fat in white adipose tissues, glucose, total cholesterol, triglycerides, LDL-c and insulin blood levels. There was an increase in HDL-c levels. No change was observed in inflammatory markers and liver enzymes. Camu-camu pulp was able to improve the biochemical profile of obesity in rats suggesting that this Amazonian fruit can be further used such a functional food ingredient in control of chronic diseases linked to obesity.

Key words: amazonian fruit, flavonoids, lipid profile, *Myrciaria dubia*, obesity.

INTRODUCTION

The prevalence of overweight and obese people is growing rapidly worldwide, so it is increasingly important to consider the role of food in the process of prevention and treatment (Alberti et al. 2009). Excess fat in the abdominal region in particular, has been linked to a metabolic syndrome that causes hypertension, dyslipidemia, and glucose

fasting. The presence of these conditions can lead to a higher risk of type 2 diabetes *mellitus* and the development of coronary artery diseases (Millán et al. 2009, Koh-Banerjee et al. 2004, Marangoni et al. 2009). Diets high in calories lead to excess body fat and diets low in calories could be an appropriate strategy to reduce excess body fat continuously and in a healthy manner (Masson et al. 2009).

The alarming increase of obesity in humans, particularly in developed countries, has demonstrated

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connection with a diet characterized by: high consumption of saturated fats and low consumption of fiber (Jenkins et al. 2009); high consumption of red meat, whole dairy products, soft drinks, refined sugars; and low consumption of fruits and vegetables (Englyst et al. 2007). With the growing interest of researchers and the general public concerning the treatment and prevention of diseases through diet, a new category of foods, called functional foods, have been extensively researched (Du et al. 2009). A food is considered functional when it exceeds the basic nutritional value and clearly contributes to the improvement of overall health and reduces the occurrence of diseases (Bell and Goodrick 2002).

In the Amazon there are numerous plant species with economic potential, among which stands out the Camu-camu fruit (Camu-camu (HBK) McVaugh). Camu-camu is from the Myrtaceae family and grows on the banks of lakes and rivers in the Amazon Forest (Yuyama et al. 2002). The interest in this fruit has increased because of its impressive quantity of vitamin C, which can reach 6 g/100 g of fresh pulp. This makes it one of the richest sources of vitamin C in the world (Yuyama et al. 2002). Camu-camu is a potential scavenger of free radicals and it strengthens the immune system. It also has other potentially important properties: it is a rich source of fiber, and it contains anthocyanins that are potent antioxidants (Zanatta et al. 2005). Camu-camu also contains significant levels of: potassium calcium, vitamin A, glucose, fructose, starch, pectin, phosphorus, and nitrogen (Silva et al. 2006, Akachi et al. 2010). These properties of the camu-camu fruit have aroused the economic and scientific interests of importers in Japan, Europe and the USA (Castañeda et al. 2008, Genovese et al. 2008).

Despite the fact that camu-camu frequently grows near the margins of lakes and rivers in the Amazon forest, there is very little information available on its biological effects and possible uses. The aim of this study was to identify the following biological effects that camu-camu pulp has in

rats: reduction of body weight, reduction of white fat tissue, and reduction of fat excreted in feces, reduction of fat in the heart and liver, changes in markers of lipid metabolism and liver enzymes, and changes in inflammatory proteins.

METHODS

GETTING PULP

The fruits were collected from the Yurican reserve located on the AM 010 highway in the municipality of Rio Preto da Eva, Amazonas, Brazil. The fruits selected were washed with chlorinated water (1.0% sodium hypochlorite). Approximately 3 kg of fruit were blanched by immersion in boiling water at 70°C for two minutes using a stainless steel basket. The cooling of the blanched fruits was performed immediately after blanching by immersing the basket containing the Camu-camu in an ice bath until it reached room temperature. The pulping process followed the recommendations of Yuyama et al. (2002).

CHEMICAL ANALYSIS OF CAMU-CAMU PULP

Measurements of total fiber, soluble fiber and insoluble fiber were performed according to the method described by Prosky et al. (1988) using enzymes Termamyl 120 L (α-amylase) with declared activity of 120 KNU/g of Alcalase 0.6 L (protease) with declared activity of 0.6 AU/g, and AMG 200 (amyloglucosidase) with declared activity of 200 AGU/ml, all manufactured by Novozymes Ltda. The enzyme activity was tested periodically using the kit Sigma TDF-C10®. The results expressed as percentage in dry matter were obtained after subtracting values of gray and white (residue of blanks corrected for ash and protein) and subtracting the crude protein (N x 6.25), N being determined by micro-Kjeldahl distillation. Additionally, were determined the composition of the samples according to the techniques described in AOAC. Quantification of quercetin was performed according to Lees and

Francis (1972). The samples were added to a solution of 95% ethanol and 1.5 N HCl were associated to obtain the extract. After obtaining the extract, the same was taken to the spectrophotometer (Shimadzu model 1601 UV-VIS).

ANIMALS AND INDUCTION OF OBESITY

The animals were from the vivarium of the INPA (Amazon Research Institute, AM, Brazil). After 24 hours of birth, pups of Wistar strain (*Rattus norvegicus* var. *Albinus*), were injected subcutaneously, in the neck, with monosodium glutamate (Ajinomoto®) at a dose of 4 mg/g body weight in a volume of 0.2 mL, during 14 days (Song et al. 2009). At 22 days old pups were weaned. During 120 days of the experiment were used 48 male rats, weighing approximately 392 g. Each animal was housed in an individual cage, with a temperature of $26 \pm 2^\circ\text{C}$ and light-dark cycle of 12 hours, receiving water and commercial chow *ad libitum* (Labina, São Paulo, Brazil). All procedures used during the experiment were according to advocating Law No. 11 794/2008 establishing procedures for scientific use of animals in Brazil.

EXPERIMENTAL PROTOCOL

We conducted a pilot project with duration of four weeks, in which it was observed that each rat consumed 25 mL of pulp served in a container that was as a medium for the experimental design. Moreover, water was served regularly. It was also observed during the pilot project the average consumption of 20 g of chow *ad libitum*, which offered throughout the project. The rats were divided into two groups with eight animals each, with equivalent weights. The first group was a non treated group (CG). The second was the experimental group that used the Camu-camu in diet (CCG), each animal received 25 mL of pulp juice Camu-camu fresh individually, daily for 12 weeks. Both groups received water and commercial chow *ad libitum* (in

pads) of Labina (São Paulo, Brazil). The animals were kept in individual cages with stainless steel castings and monitored weekly with the ingestion of food and water. Below the cages were placed in trays paper for packaging remains of diet and feces. Water and food were offered in nipple drinker's glass, metal and glass jars, respectively. The water and feed containers were filled every 2 days and the intake assessment was performed weekly. The trays containing the diet remains were collected weekly and dried at 60°C for 15 minutes. After drying, the feces were separated from the diet remains and weighed separately.

PREPARATION OF SAMPLES

At the end of 12 weeks, the rats were fasted for 12 hours, than were weighed and anesthetized by overdose of choral hydrate i.p., suffering sacrifice in the same period, while blood samples were drawn at the heart pulse and stored in tubes containing sodium heparin (125 U.L^{-1}) and kept on ice totaling 5 mL of blood. After this procedure, they centrifuged at 3,000 rpm for 5 minutes at 4°C , plasma was collected and stored in a freezer at -80°C until realization of dosages biochemical determinations. The white adipose epididymal and visceral fat, liver and heart were removed and weighed immediately after the sacrifice.

BIOCHEMICAL ANALYSIS

Plasma insulin was determined by solid phase radioimmunoassay, using kit Coat-A-Count (DPC) in a gamma counter (RiaStar™) - (Gamma Counting Systems) for counting of radioactivity. The dosage of leptin was performed using the kit DLS test 23 100® for Active imunorradometric. The tumor necrosis factor- α (TNF- α) test was performed by the enzyme-linked immunosorbent assay by using kits from Biosource International Cytoscreen™ as the vendor's manual. The level of C-reactive protein (CRP) was accomplished using the "kit" N latex mono, manufactured by Dade Behring Marburg GmbH® (Germany). Plasma levels of total

cholesterol and triglyceride fractions were determined by enzymatic colorimetric method using commercial kits Labtest®. After precipitation of LDL (low density lipoprotein) and VLDL (very-low-density lipoprotein) phosphotungstic acid/MgCl₂, HDL (high density lipoprotein) cholesterol was calculated as the difference between total and HDL cholesterol. For measures of glucose, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymatic kits were used Labtest® (Minas Gerais, Brasil) and readings were made in automated equipment Cobas Mira Plus (Roche®) (Kondoh et al. 2009, Brandt et al. 2009). The extraction of fat tissues was performed in triplicate samples using the Soxhlet extract with intermittent use of organic solvent (ether), following for total lipid determination (Jen et al. 1981).

STATISTICAL ANALYSIS

Data were expressed as the mean and standard deviation. The normality of data was analyzed using the Kolmogorov-Smirnov test. Data with a normal distribution were analyzed by one-two-way ANOVA for factors with and without a supplemental post hoc Tukey test was used to determine significant differences between the two groups. Observed data in which the distributions were not considered normal were tested using nonparametric Kruskal-Wallis. The findings of the results collected were made by the statistical program 'R' version 6.0. FEZ-SR (Manaus/Amazonas/ Brazil). A significance level of $p \leq 0.05$ was adopted for all analysis.

RESULTS

Chemical properties of Camu-camu pulp were showed in the Table I. The Camu-camu pulp showed a high level of quercetin (400 mg/100 g) and low content of soluble and insoluble fibers, 0.73 g/100 g and 0.48 g/100 g, respectively. The pH observed was 2.64 and ascorbic acid contents were 864 mg/100 g.

TABLE I
Chemical composition and physical chemical characteristics of Camu-camu pulp. (g/100g).

Parameter	Content
Humidity (g/100g)	92.65±0.03
Lipids (g/100g)	0.05±0.01
Protein (g/100g)	0.29±0.01
Reducing sugar (g/100g)	4.4±0.03
pH	2.64±0.01
Soluble solids (Brix)	6.20±0.00
Acidity (g/100g)	3.40±0.06
Ascorbic acid (mg/100g)	861.73±64.13
Anthocyanins (mg/100g)	9.98±0.19
Insoluble fiber (g/100g)	0.48
Soluble fiber (g/100g)	0.73
Total dietary fiber (g/100g)	1.23
Quercetin (mg/100g)	400.0

The effects of the supplementation of Camu-camu pulp in obese rats were realized during four week of treatment. The supplementation with Camu-camu pulp showed reduced body weight in the CCG group (31.7%) when compared with CG (Table II). A reduction in the weight of visceral tissue (36.4%) and epididymal tissue (24%) in the CCG group also was observed, while in the CG group the weights of this tissue increased, 14.3% and 20.2%, respectively. An increased was also observed in the feces (50%) and liver (140%) in CCG group in comparing the CG group.

The treatment of obese rat with Camu-camu pulp also reduced cholesterol (39.6%) and triglycerides (40.6%), in comparing with increase observed of cholesterol and triglycerides in the CG group, 60% and 44%, respectively (Table III). LDL (2.14%) and VLDL (36.4%) also were reduced in obese rats treated with Camu-camu pulp, when compared with increase observed in the CG group, 118% and 14.3%, respectively. Glucose also was reduced in obese rats (23%), while a increase of 19.4% was observed in control group intake with chow ad libitum.

TABLE II
Quantitative analysis of weight-weight of the heart, liver, visceral fat, epididymal fat, index of Lee, feces, liver and heart in obese rats treated with Camu-camu juice.

Variables	CG			CCG		
	Initial	Final	P	Initial	Final	P
Body Weight (g)	402. ± 25.8	309 ± 27.2 ^(a)	0.005	435.9 ± 8.9	297.3 ± 34.4 ^(b)	0.0005
Weight index (cm)	39.4 ± 1.2	33.3 ± 26.4 ^(a)	0.02	38.2 ± 1.2	35.6 ± 0.92 ^(a)	0.0001
Feed intake (g)	122.0 ± 7.1	137.0 ± 2.9 ^(a)	0.003	120 ± 7.1	137 ± 2.9 ^(a)	0.03
Visceral fat (g)		16.0 ± 2.8 ^(a)			27.1 ± 4.4 ^(b)	
Epididymal fat (g)		18.0 ± 5.2 ^(a)			25.5 ± 5.1 ^(b)	

CG=control group; CCG= Camu-camu group. Values are mean± SD (n =8). Data were tested by two-way ANOVA; when interactions were significant (P<0.05). Tukey'spost hoc test was performed to determine the specific differences between mean values. Kruskal Wallis Test for medium periods (p-value ≤ 0.05), where groups with the same letters are not significant.

TABLE III
Lipid and glucose profiles in obese rats treated with Camu-camu juice.

Variables (mg.dL -1)	Experimental group						P value
	CG			CCG			
	Initial	Final	Δ=%	Initial	Final	Δ=%	CGxCCG
Cholesterol	53.1 ± 10.9	85.2 ± 17.0 ^(a)	60.0	78.2 ± 14.8	47.2 ± 26.1 ^(b)	39.6	0.0011
Triglycerides	222.0 ± 4.8	320 ± 12.3 ^(a)	44.0	222.0 ± 1.7	131.8 ± 11.7 ^(b)	40.6	0.0005
HDL	36.8 ± 0.33	29.8 ± 1.2 ^(a)	19.0	33.3 ± 0.48	37.4 ± 3.8 ^(b)	12.3	0.0008
LDL	68.8 ± 3.7	150.2 ± 4.1 ^(a)	118.0	69.8 ± 15.3	68.3 ± 32.5 ^(b)	2.14	0.0012
VLDL	23.7 ± 8.4	27.1 ± 8.8 ^(a)	14.3	25.5 ± 6.1	16.2 ± 10.8 ^(b)	36.4	0.0023
Glucose	210.2 ± 4.2	250.5 ± 1.3 ^(a)	19.4	220.8 ± 3.4	170.3 ± 2.8 ^(b)	23.0	0.0001

CG=control group; CCG= Camu-camu group. Values are mean± SD (n =8). Data were tested by two-way ANOVA; when interactions were significant (P<0.05). Tukey'spost hoc test was performed to determine the specific differences between mean values. Kruskal Wallis Test for medium periods (p-value ≤ 0.05), where groups with the same letters are not significant.

Biochemical analysis showed an increase in the activities of AST, ALT and ALP, 37.1%, 104% and 10.7%, respectively (Table IV). A reduction of 44.5 % in the insulin activities was observed, as well as a reduction in the levels of TNF-α (12.7%) in obese rats treated with Camu-camu pulp.

DISCUSSION

High intakes of plant foods (i.e. vegetables, legumes and fruits), which contain flavonoids and polyphenolic compounds, have been directly

associated with the management and prevention of obesity, type 2 diabetes *mellitus* and other cardiovascular diseases. Quercetin is a major flavonol abundant in plant product, and has been reported to possess antioxidative, anti-inflammatory and lipid-regulating properties (Kim et al. 2012). The physical-chemical properties of pulp of camu-camu, as expected showed high moisture and low lipid contents (Table I). pH observed was 2.64, which can be explained by the ascorbic acid content (864 mg/100 g). The Camu-camu pulp also

TABLE IV
Biochemical and inflammatory profile of obese rats treated with Camu-camu juice.

Variables	Experimental group			P value
	CG	CCG	Δ=%	CGxCCG
Insulin	12.8 ± 0.8 ^(a)	7.1 ± 0.8 ^(b)	44.5	0.0011
Leptin (ng/mL)	15.4 ± 0.6 ^(b)	13.4 ± 1.9 ^(b)	12.9	0.1115
TNF-α (pg/mL)	276.4 ± 48.6 ^(c)	241.1 ± 4.1 ^(b)	12.7	0.1315
CRP (g/L)	5.2 ± 4.8 ^(a)	4.2 ± 7.7 ^(a)	19.2	0.8228
AST (U/l)	105.0 ± 0.8 ^(a)	144.0 ± 88.8 ^(a)	37.1	0.1210
ALT (U/l)	22.0 ± 27.9 ^(b)	44.9 ± 33.6 ^(a)	104.0	0.0022
ALP (U/l)	25.2 ± 15.4 ^(a)	27.9 ± 20.3 ^(a)	10.7	0.2211

CG=control group; CCG= Camu-camu group. Values are mean± SD (n =8). Data were tested by two-way ANOVA; when interactions were significant (P<0.05). Tukey'spost hoc test was performed to determine the specific differences between mean values. Kruskal Wallis Test for medium periods (p-value ≤ 0.05), where groups with the same letters are not significant.

showed a level of 400 mg/100 g of quercetin and low content of soluble (0.73 g/100 g) and insoluble fibers (0.48 g/100 g).

Quantitative analysis of weight-weight of the organs of obese rats treated with camu-camu juice showed a reduced body weight in the CCG group while in the CG group there was no reduction in this variable. There was a reduction in visceral tissue and epididymal tissue in the CCG, while in the CG the weights of these tissues are enlarged compared to the experimental group (Table II).

These beneficial effects may be due to the combination of the properties of phenolic compounds present in high concentrations (1.89 g/100 g) diet imposed. Kwon et al. (2007) observed the effect of extracted from black soybean on body weight, weight of white adipose tissue and lipids in rats fed a high fat diet. In this study, rats fed the diet containing 10% soybean black, equivalent to 0.037% of phenolic compound, body weight and white adipose tissue was significantly reduced when compared with control rats. In another study (Song et al. 2009) looked at the effects of extracts from *Rhizoma Dioscoreae Tokoronis* (RDTEs) on plasma

lipids, body weight and lipogenic enzymes in rats given a standard diet with 60% fat, while another group received the same diet further increased with 40% RDTE. In the rats of second group were found body weight reduction in epididymal adipose tissue. But when used with yellow tomato lycopene, any positive effect was not observed on reducing body weight and white adipose tissue (Gitenay et al. 2007). The reduction of body fat and hypolipidemic effects of diet formulated Fatclean were examined in Sprague-Dawley rats fed with a high fat diet; a group of control animals received a normal diet, while the second group received a diet with an increase of 15% fat and a third group beyond the 15% fat receiving Fatclean 5% for 6 weeks. The diet contained a formula Fatclean of phenolic compounds (14.3 mg/g) and other functional compounds. The group which held Fatclean diet significantly reduced the final body weight and visceral fat (Woo et al. 2009).

The CCG group also had an increased of the amount of fat eliminated in feces (50%) and liver (140%) (Table II). This may be related to reduction of cholesterol (39.6%) shown in Table III in the

CCG, as a result of the presence of dietary fiber in the pulp of Camu-camu (Table I). Fiber feeding is associated with bile, promoting a blockage in your intestinal reabsorption, resulting in the fecal excretion (elimination through feces), stimulating the liver to manufacture bile again from the blood cholesterol, which makes its reduction (Howarth et al. 2001, Katcher et al. 2008).

In the analysis of the lipid profile CCG reduced total cholesterol, triglycerides, increased HDL and reduced VLDL. Glucose was also reduced during observation period, when compared to CG group as presented in Table 3. Polyphenol-rich foods and beverages such as red wine, green tea and apples have been associated with increased HDL, reduced intestinal absorption of dietary lipids, interfering with its emulsification, digestion, and micellar solubilization (Katcher et al. 2008, Kovacs and Mela 2006). Hypotheses, which can be seen on the mechanism by which the Camu-camu is responsible for the decreased levels of concentrations of variables of lipid profile, suggesting that some nutrients given by this fruit that rise this effect are the increased excretion of cholesterol in the form of bile acids, due to redistribution of concentrations in plasma and tissues; or the increase in liver LDL receptors, leading to a decrease in plasma concentrations (Koo and Noh 2007). It is also suggested that the fruit is able to inhibit the synthesis in the liver and or accelerate the catabolism of VLDL and chylomicrons, increased activity of the enzyme lipoprotein lipase (LPL), which could result in a reduced plasma triglycerides, mainly in the postprandial state (Ros and Mataix 2006).

Another important factor reported in the literature is large concentration of fiber, in which the fruit of the Camu-camu is high (Silva et al. 2006). This concentration may have helped to reduce the levels of insulin and glucose in rats of the group CCG, improving glycemic control, which consequently reduces the need for insulin. Soluble fibers are the most effective in controlling

blood sugar and it is known that there is a property related to the ability of slowing down gastric emptying, providing openings for the penetration of carbohydrates within the fiber by reducing the amount available for absorption, and no contact with the intestinal mucosa, reducing blood glucose levels, which promotes hormonal changes (Joshi et al. 2001). The soluble fiber is effective in reducing postprandial glycemia in both experimental studies and in healthy subjects and diabetic patients related to reduce absorption of glucose, due to the increased viscosity of intestinal contents (Kwon et al. 2007).

The presented results in CCG indicating that the pulp caused an increase in the activities of AST, ALT and ALP (Table IV), which may be indicative of liver injury or toxicity of the fruit. Since the goal of this experiment was to observe not the result in liver toxicity, but the way the liver responds to the ingestion of the fruit, it will be important for future research to verify these effects, including biochemical analysis beyond the possibility of using a biopsy of the organ to confirm these findings. In this study there was a reduction in the CCG insulin levels. In our experiment it was observed that the pulp of camu-camu holds a certain amount of dietary fiber (Table I) which may have account for reducing blood glucose levels (Table III) in rats of this group, improving glycemic control, which consequently reduces insulin requirements (Table IV). There was a reduction in the CCG a concentration TNF- α . The main sources of TNF- α are visceral and subcutaneous adipose tissues (Howarth et al. 2001). These tissues were reduced in the group that ate the flesh of the Camu-camu which contributed to the reduction of this cytokine (Table IV). However, Katcher et al (2008) revealed narrower molecular bond between TNF- α and obesity, with the increase of the TNF- α expression in obesity, decreases with weight loss and improving insulin sensitivity.

CONCLUSIONS

This investigation can conclude that the pulp of Camu-camu slices have nutrients that may explain the observed effects in our experiments, which collaborated with the reductions in body weight and epididymal and visceral fat of reducing some markers of lipid metabolism, reducing fat deposited in the feces, heart and liver, in addition to decrease the inflammatory proteins. Therefore, the results of this fruit's action will serve as a basis for future studies on prevention or treatment of obesity and the relationship of using the same diet with the aim to reduce the risk factors of diseases associated with obesity.

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RESUMO

O fruto Camu-camu da amazônia (*Myrciaria dubia* HBK Mc Vaugh) apresenta um interesse atrativo na alimentação e indústria de cosméticos devido ao rico conteúdo de Vitamina C, flavonoides e antocianinas. O objetivo deste estudo foi investigar a ação antiobesidade da ingestão da polpa de Camu-camu em modelo de ratos obesos. Ratos Wistars foram induzidos a obesidade pela injeção subcutânea de glutamato monossódico recebendo dieta *ad libitum*. Os ratos foram divididos em dois grupos. Um grupo experimental, que ingeriram 25 mL/dia de polpa de Camu-camu (CCG) e um grupo não tratado (CG). Após 12 semanas, os animais foram sacrificados.

Sangue, fígado, coração e tecido adiposo foram coletados e pesados, assim como determinado os perfis bioquímicos e inflamatórios. Animais que receberam a polpa de Camu-camu reduziram o peso do tecido adiposo, os níveis plasmáticos de glicose, colesterol total, triglicerídios, LDL-c e níveis de insulina no sangue. Houve um aumento nos níveis de HDL-c. Nenhuma mudança foi observada nos marcadores inflamatórios e enzimas do fígado. A polpa de Camu-camu foi capaz de melhorar o perfil bioquímico da obesidade em ratos, sugerindo que este fruto da Amazônia pode ser futuramente utilizado como um ingrediente alimentar funcional no controle de doenças crônicas ligadas a obesidade.

Palavras-chave: fruto da Amazônia, flavonoides, perfil lipídico, *Myrciaria dubia*, obesidade.

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