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Heavy Metals in Processed Products of Acai (*Euterpe precatoria*) and Cupuassu (*Theobroma grandiflorum*) in the Western Amazon Region of Brazil

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ABSTRACT

Background: Acai and cupuassu are fruits from Amazonian trees which have important role in human nutrition in this environment. The contamination of food products by heavy metals must be inspected in order to avoid healthy problems to the consumers. During processing and preparation, foods stay for a significant period of time in contact with industrial containers and utensils. This contact can lead to changes in the nutrient composition or even change flavor and color of the products. These changes can be related to the migration of elements present in the composition of the equipment used in the production process. There are few studies that make an assessment of toxic elements in food products, particularly concerning native Amazonian fruits. **Objective:** The aim of this study was to evaluate the concentration of potentially toxic inorganic compounds in products of acai and cupuassu processed at the Joint Consortium for Economic Reforestation (RECA) in the state of Rondônia, in the Western Amazon region of Brazil. **Results:** It was observed in the analysis of the elements chromium, copper, manganese, nickel, lead and zinc that they were present in all the evaluated products – acai pulp, jam and jelly, and cupuassu pulp, jam, jelly without fruit pieces and jelly without fruit pieces. However, in comparison with the levels established by the Brazilian legislation, it was possible to observe that none of them exceeds the determined levels. **Conclusion:** Although present, the levels of the elements found in the evaluated products were considered low. The concentrations found imply no risk to the health of the population.

INTRODUCTION

The Amazon region is characterized by the availability of fruits with high economic and nutritional potential. The interest in these fruits increases daily - nationally and internationally - mainly due to their exotic flavors and aromas. The use of natural products in the North of Brazil is characterized by the lack of regulation and industrialization. The determination of nutritional and functional aspects of these products will extol their virtues as human food and aid in preventing nutritional deficiencies. However, little is known about the actual composition of the mineral content in foods from these native fruits. Some microelements are essential to human health, but the limits of tolerance to them can be very low – meaning that they can become toxic at certain levels (Leterme *et al.*, 2012; Naozuka and Oliveira, 2007). According to World Health Organization, metallic elements can be stored in the tissues and organs (Molina *et al.*, 2015). Heavy metal pollution is identified as one of the

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consequences brought about by development and economic progress and this pollution brings a global concern especially that possible consequences occur even in exposures to small concentrations (Guzman *et al.*, 2016).

Acai (*Euterpe precatoria*) is a palm tree very common to Western Amazon (Amazonas, Acre, Rondonia, and Roraima states). It propagates by seeds rather than by tillers – unlike many palm trees. Its fruiting occurs mainly at the beginning of the year, during the Amazonian winter. The fruits are round, purplish, almost black when ripe, with rich edible pulp (Galotta and Boaventura, 2005; Sanabria and Sangronis, 2007; Santana and Jesus, 2012) It is popularly appreciated by the Amazonian population and plays an important socioeconomic and cultural role. The fruits are widely used in local cuisine as juice - traditionally known as "acai wine" - in meals with cassava flour or tapioca flour in combination with meat, salted fish or shrimp and mixed with fruit and granola. It makes excellent jams, jellies, chocolates, creams, ice creams, and liqueurs (Schirmann *et al*., 2009; Yuyama, *et al.*, 2011). Acai has a high caloric content and since it has a considerable level of iron, it is empirically used to treat cases of anemia throughout the Amazon region (Yuyama *et al.*, 2002; Toaiari *et al*., 2005). Nowadays, demand for acai is growing in the international market. It is considered as an exotic fruit with high levels of lipids, anthocyanin and polyphenols - substances with high antioxidant capacity and proven beneficial health effects (Sangronis *et al*.; 2006; Schirmann *et al*., 2009).

Known popularly as cupuassu, *Theobroma grandiflorum* Sachum. belongs to the Sterculiaceae botanical family and is one of the most attractive fruits of the Amazon region, due to the organoleptic characteristics of taste and intense aroma of the pulp (Ferreira *et al*., 2005; Araujo, 2007). The harvesting occurs after the fruits fall from the tree, when the dry leaves on the ground protect the fruits avoiding the breaking of the shell. There are three varieties: cupuassu-round (most common), cupuassu mamorama (more elongated) and cupuassu-mamau (round shape, without seeds) (Villachica *et al*., 1996; Brasil, 2015). The utilization of the components of these fruits is maximized – the woody hard shell can be used as cattle feed and fertilizer, or in packaging for gifts; the pulp is used in the preparation of ice creams, juices, jams, liqueurs, pastries, mousses, creams, chocolates, candies, biscuits and yoghurts; the seeds, once dried, are used in the manufacture of high quality white chocolate. The oil extracted from the seed is used in the cosmetics industry (Costa *et al*., 2003; Rogez *et al*., 2004). Cupuassu presents several important constituents for human nutrition. The pulp has significant amounts of pectin (soluble dietary fiber), calcium, iron, magnesium and vitamin C, but low levels of proteins and lipids. Stearic, oleic, linoleic, and arachidonic acids are the main fatty acids of the seed. The natural acidity of the pulp and the high content of pectin are characteristics that allow the production of juices, jams, candies, and jellies - prolonging the storage of the products (Villachica *et al.*, 1996; Araujo, 2007).

During the processing and preparation, foods stay for a significant period of time in contact with industrial containers and utensils which can lead to changes in the nutrient composition or even change flavor, aroma and color. These changes can be related to the migration of elements present in the composition of the equipment used in the production process. This transfer of elements can be positive when they are scarce in the population diet, or negative, when there is a release of heavy metals in amounts which may have adverse effects on health. The main factors affecting the migration of elements from the equipment to food are the pH level of the food, the period in which the food remains in contact with the container, and the water content of the preparation. Temperature, contact area, agitation, and the presence of chelating agents influence to a lesser extent the dissolution of metals (Quintaes *et al*., 2004; Quintaes, 2006; Dantas *et al*., 2007).

Some minerals have essential organic functions, acting both in ionic form and in the constitution of organic compounds - such as enzymes (catalysts), hormones, secretions and proteins of organic tissues (structural function). They are classified as essential minerals when they perform vital functions in the body, or when their reduction or deficiency leads to changes in one or more physiological functions. Some trace elements are considered not essential because they are not proven necessary in the human diet. Minerals that are potentially toxic are trace elements can be toxic at high concentrations, although some of them are used in vital metabolic reactions, but in very low concentrations (Bianchi *et al*., 2006; Pacheco, 2006).

The toxicity of minerals depends on the dose or exposure time to the physical and chemical form of the element and the means of absorption. The toxic character of the mineral depends on the interaction with the human body, its chemical form and physiological effects in organs or systems. There are several minerals considered toxic when consumed in large amounts. Among them are Hg, Cd, Pb, As, Mn, Cr, Ni, Se, Te, Sb, Be, Co, Mo, Sn, Cu, and Pb. These minerals can act with ligands, diffusers, and macromolecules present in membranes. This property can lead to bioaccumulation, biomagnification along the trophic chain, persistence in the environment, and dysfunction in metabolic processes. Bioaccumulation and biomagnification convert normal concentrations into toxic levels to humans (Tavares and Carvalho, 1992; Maihara *et al*., 2016).

Among the mentioned minerals, some are considered essential for metabolic activities in the human body, but in low concentrations. Above certain levels, they become potentially toxic. Therefore, an essential trace mineral can be a contaminant when found in food above nutritionally desirable levels. In particular chromium (Cr) is required for the metabolism of fat, cholesterol, and glucose. Copper (Cu) is a component of enzymes such as tyrosinase, cytochrome oxidase and superoxide dismutase and is significant for hemoglobin synthesis and bone formation. Manganese (Mn) - cofactor of arginase – is present in metalloproteins as pyruvate decarboxylase. Nickel (Ni) participates in the regulation of lipids and synthesis of phospholipids. Zinc (Zn) is an antioxidant that acts in the immune response component of several metalloenzymes and in the biosynthesis of proteins and nucleic acids. However, all of these minerals, when in high concentrations, may result in damage to the human body. On the other hand, lead (Pb) is considered exclusively toxic because it changes antioxidant activity by inhibiting functional SH group in many enzymes. (Brasil, 2003; Tirapegui, 2006; Schwanz *et al*. 2008; Philippi, 2008).

In food industry, the equipment used for pulping and production of jams and jellies is made mostly of stainless steel due to its durability and quality. Stainless steel is a combination of iron with chromium and nickel metals in the approximate amounts: zero to 30% nickel, 11 to 30% chromium and 50 to 88% iron. There may be small quantities of manganese and copper present. It is noteworthy that the Fe3 + and Cr3 + are often scarce in diets and populations rarely suffer from their excess. Nickel can be toxic depending on the amount ingested 25µg/g) (Quintaes, 2000; Tomita, 2006).

There are few studies related to toxic compounds present in processed foods from Amazonian fruits. This study aimed to evaluate the concentrations of Cr, Cu, Mn, Ni, Pb, and Zn in pulps, jams and jellies produced from acai (*Euterpe precatoria*) and cupuassu (*Theobroma grandiflorum*) processed at the Joint Consortium for Economic Reforestation (RECA), in the state of Rondônia, Western Amazonia.

MATERIALS AND METHODS

The cupuassu and acai products were collected at the Joint Consortium for Economic Reforestation (RECA), located at BR 364, km 355.5, District of Nova California, in Porto Velho, Rondônia. The frozen fruit pulps were transported in plastic bags and packed in ice in polystyrene boxes to the Environmental Biogeochemistry Laboratory Wolfgang C. Pfeiffer of the Federal University of Rondônia (LABIOGEOQ/UNIR). The jams and jellies (with and without fruit pieces) were transported at room temperature, as they are marketed, in 500 mL glass bottles sealed with metal caps. All the collected samples had different dates of production. During the pulping of acai at RECA three parts water are added to one part pulp. The water comes from wells and undergoes treatment (Martins, 2002). Based on the IN01 classification (Brasil, 2000), the RECA acai fruit is considered Type B (medium fruit). During the pulping of cupuassu no water is added. There are two types of jelly of cupuassu. One is without fruit pieces - in which the jelly is sieved to give a semi-liquid product. The other is jelly with fruit pieces - without being strained. In this study the products examined were acai pulp, jam and jelly, and cupuassu pulp, jam, jelly with fruit pieces and jelly without fruit pieces.

The preparations of jams and jellies of acai and cupuassu are processed in baker stainless steel, heated by liquefied petroleum gas. The jelly of cupuassu is prepared in cast aluminum pans on a wood burning stove. The proportions of fruit pulp, sugar, water and pectin in the products are shown in Table 1.

At the laboratory, 100 grams of the pulp samples were weighed in triplicate and stored in sterile plastic bags. The bags were sealed and frozen in a freezer at -18°C. The jam and jelly samples were kept in their original containers, stored at 4°C. The wet samples were weighed (15.0 grams of each sample) and dried in an oven (COEL-HM) at 60°C for 48 hours. After that, they were calcinated in a muffle (EDG 3000 model), at the initial temperature of 60°C for 15 minutes, gradually raising the temperature to 450°C which was maintained for a period of 24 hours to complete calcination of the sample. After calcination, the samples were then acidified in an open digestion system, adding 4.0 mL HNO_3 in accordance with the methods #393 and 394/IV described by IAL (IAL, 2008). The beakers were placed on a heating plate at 150°C until the liquid was evaporated. Suspension was achieved with 6.0 mL of concentrated HCl. Again the beakers were placed on a heating plate at 150°C until the liquid was evaporated. 20.0 mL of 1N HCl was added to the material and the final solution was filtered through filter paper (Whatmam 44 - white band) and packed for a final volume of 20.0 mL in Falcon tubes with lids. The samples were registered and stored under refrigeration (4°C) until the minerals were analyzed. The minerals were quantified by flame atomic absorption spectrophotometer (Perkin Elmer model AAS400) calibrated with standard solutions of Fe, Zn, Mn, and Cu (IAL, 2008). The results were expressed in µg mineral / g samples.

To calculate the weight in grams of the minerals, the following formula was used:

$$\text{mineral concentration in the sample } (\mu g/g) = \{[(S - C) \times FV] / M\}$$

where S = concentration of the sample (µg/mL); C = average concentration of control solution (µg/mL); FV = final volume of the sample (mL); M = initial mass of the sample (g).

The glassware was previously washed and immersed in a detergent solution of 5% Extran (Merck) for 24 hours and then rinsed in running water. The material was kept immersed for 48 hours in a solution of 5% HNO_3 and then rinsed twice with distilled water. Then it was dried in an oven for 24 hours at 50°C. After that, the materials were cooled in a desiccator containing silica gel for 30 minutes (Bastos *et al*., 1998).

To ensure analytical quality control, all analyses were done using a pool consisting of three samples from different dates of production, which were mixed to compose the final sample. Analyses were performed in triplicate with a control also in triplicate.

In the analysis of minerals, a certified reference sample of the International Atomic Energy Agency was used (IAEA-392 Algae) (IAEA, 2005), serving as a reference for measuring the quality of the process. This reference material was obtained from the algae *Scenedesmus obliquus* 208.

For evaluation of the mineral content a completely randomized design it was used with three replications of three samples. All data was subjected to analysis of variance. The standard deviation and coefficient of variance were calculated for each mineral content. Statistical analyses were performed with the BioEstat program, 5th edition, 2007.

RESULTS AND DISCUSSION

The values obtained in the control samples of analyses showed low coefficients of variance and standard deviation, proving that the analyses were conducted with technical rigor, which gives reliability to the study.

The inference on the accuracy of the methods used in this study by using the reference certified sample IEAE-392 Algae (IAEA, 2005), showed that the recovery percentages for Cr, Cu, Mn, Ni, Pb, and Zn ranged from 78-115%.

In Table 2 are shown the amounts of chromium, copper, manganese, nickel, lead, and zinc in the products. Chromium concentration was higher in acai pulp with 0.70 µg/g followed by acai jam with 0.40 µg/g. In relation to cupuassu, the concentration of 0.1 µg/g was found in all products. Santana and Jesus (2012), studied acai fruit collected at the Industrial Complex of Manaus, Brazil, and found 3.10 µg/g. Quintaes *et al.* (2004) studied the migration of chromium from stainless cookware to an 0.88 M acetic acid solution and observed values from 0.01 to 0.03 µg/g chromium. According to these authors these values are not considered toxic to human health. To be considered as toxic, chromium has to be ingested in doses above 100 µg/g and even an acid medium (pH <7.0) is necessary to convert $Cr3+$ into $Cr(OH)6$, which is toxic and carcinogenic (Tomita, 2006; Silva *et al.*, 2016). According to Ngadi *et al.* (2015), the main human sources of chromium are electroplating, leather tanning and metal-fishing plant industries.

In relation to copper in acai products, concentrations of 2.40, 1.70, and 1.20 mg/g were observed respectively in pulp, jam and jelly. Regarding cupuassu, jam without fruit pieces showed the highest value, 5.60 mg/g, while pulp showed 1.20 mg/g, jam 0.90 mg/g and jelly with fruit pieces 1.30 mg/g. Examples of fruits that have high concentrations of this mineral include raw Bahia coconut, with 4.20 mg/g and tamarind with 2.90 mg/g. Nuts in general have high levels of copper such as cashew nuts with 19.20 mg/g and Brazil-nuts with 17.90 mg/g in the edible portion (NEPA, 2011). Ordinance No. 685 of the Ministry of Health determines that copper is considered toxic above 1000 µg/100 g in fruits and vegetables. It was observed that in all studied products, the values are below levels established by the legislation. According to Aziz *et al.* (2015), certain metals such as Fe, Zn, Mn and Cu are essential at low level, other metals like Cd, Cr, Pb and Ni are toxic and may pose a great danger to humans.

The incidence of toxicity by copper in humans is rare. The ingestion of great quantities of copper salts can cause acute intoxication. Dangerous quantities can be ingested from acid foods that were kept for long periods of time in contact with metallic containers. Chronic intoxication is more frequent but can be attributed more to failure in excretion in the bile than to excessive intake. This type of intoxication is characterized by gradual accumulation in the liver which can result in damage of this organ. The bioavailability of copper is affected by thermic treatment due to formation of products from Maillard reaction (amino sugars). These compounds reduce the number of available sites for the formation of bonds between metals and nitrogen, resulting in the decrease of organometallic compounds of high bioavailability (Hashimoto *et al.*, 2016; Cozzolino *et al.*, 2008).

In respect of manganese, pulp, jam and jelly of acai had 45.20 µg/g, 37.50 µg/g and 40.90 µg/g respectively. In the cupuassu products, low concentrations of Mn were observed. The highest value was 3.20 µg/g in pulp. Yuyama *et al.* (1997) found 1.75 µg/g in cupuassu pulp while NEPA (2011) determined 2.00 µg/g. Santana and Jesus (2012) found 6.60 µg/g in acai fruits. It is recommended 18.00 µg per day for men and 23.00 for women. The limit to avoid adverse effects is 150.00 µg per day (Silva *et al.*, 2016). All concentrations found in this study are low and do not cause risk to human health. Moreover, Mn has a low absorption level in the bowel, only 5% of the ingested quantity. Toxicity symptoms can appear in months or even years. They are: lack of appetite, reproductive problems, anemia, ataxia and damage to the neurological system (Tomita, 2006).

The highest values of nickel found in the analysis were 0.50 µg/g in acai pulp and 0.30 µg/g in both acai jam and jelly. In relation to cupuassu, the highest concentration was 0.1 µg/g, found in pulp, jam and jelly without fruit pieces. Quintaes *et al.* (2004) found the migration of 0.01 to 0.21 µg/g nickel into the medium. Fernandes *et al.* (2007) found from 0.01 to 1.53 µg /g dry matter of Ni in corn grain. Schwanz *et al.* (2008) found values of 0.77 to 4.31 µg/g in samples of *Peumus boldus* and this concentration was not considered toxic because it is below the recommended amount of between 5.00 and 15.00 µg per day. Migration of Ni during food preparation in stainless steel utensils is small. This element can be found in higher concentrations in canned foods, sugars and preservatives (Quintaes, 2000). Toxicity for Ni is unlikely, due to the homeostatic regulation. When it occurs, one of the intoxication symptoms can be gastrointestinal irritation due to excretion of Ni salts. Deficiency of certain nutrients such as iron, zinc, copper and ascorbic acid influence some metabolic activities (Tomita, 2006).

Lead concentrations were considered low, since the acceptable limits of Pb in foods range from 0.05 to 2.00 µg/g as described in Ordinance No. 685 (Brasil, 1998). The highest value found in this study was 0.08 mg/g in cupuassu jelly with fruit pieces. In relation to acai, the concentration of 0.01 µg/g of lead was found in all products. Santana and Jesus (2012) found 16.00 µg/g Pb in acai fruits. Fernandes *et al.* (2007) found values from 0.00 to 331.60 µg/g Pb in dry corn grain. Quintaes *et al.* (2004) found that the migration of Pb from stainless steel pans to a 0.88 M acetic acid solution was not significant, and concluded that cooking in stainless steel equipment is safe to human health. Lead is found widespread in the environment and in foods and beverages in the form of metallic lead, ion, inorganic salts and organometallic compounds. Because it is very dense and water soluble it is easily absorbed by the human body. This element has no essential function in the body, but has many adverse effects. When ingested, absorption occurs in the bowel, and is transported to the hemoglobin in red cells, which deposit it in the bones. Lead crosses the blood brain barrier, acting on the nervous system and can cause ataxia, convulsions and coma, depending on the level of intoxication (Tavares and Carvalho, 1992; Maihara *et al.*, 2016; Schwanz *et al.*, 2008).

Among the acai products, pulp had the highest concentration of zinc, 2.30 µg/g. Among the cupuassu products, the highest concentration was observed also in pulp, 1.40 µg/g. Santana and Jesus (2012) found 1.00 µg/g in acai fruits. Fernandes *et al.* (2007) found values from 8.84 to 115.36 µg/g in corn grain dry matter. Nogueira *et al.* (2008) found 15.00 to 100.00 µg/g also in corn grain dry matter. Quintaes *et al.* (2004) observed migration of Zn during the cooking from iron pots to a 0.88M acetic acid solution, from 4.80 to 2.80 µg/g. According to Cardoso (2006), Zn has been considered a relatively nontoxic mineral because only very high levels of intake (1,500 µg per day) can cause nutritional imbalance in the human body. A high intake of zinc can induce copper deficiency, leading to damage in some metabolic activities. Symptoms of zinc toxicity include anemia (induced by copper deficiency), vomiting, diarrhea, depletion of immune function and kidney injury.

Table 1: Proportions of pulp, sugar, water and pectin in fruit pulps, jellies and jams of acai and cupuassu produced by RECA.

Product	Pulp	Sugar	Water	Pectin	Procedures
Acai jam	1	1	0	0	Defrosting of the fruit pulp and addition of sugar. Cooking of the product until desired consistency.
Acai jelly	1	0.5	0	0.004	Blending and sieving of the pulp. Addition of sugar. Utilization of pectin to achieve consistency.
Cupuassu jam	1	1	0.05	0	Blending pulp and water and addition of sugar. Cooking of the product until desired consistency.
Cupuassu jelly without fruit pieces	1	0.5	0.2	0	Boiling pulp with water, blending and sieving. Addition of sugar and boiling until desired temperature.
Cupuassu jelly with fruit pieces	1	0.7	0.2	0	Blending pulp and water, without sieving. Cooking the product until desired consistency.

Table 2: Amounts of chrome, copper, manganese, nickel, lead, and zinc per gram of processed products from cupuassu and acai.

Elements	Products						
	Acai pulp	Acai jam	Acai jelly	Cupuassu pulp	Cupuassu jam	Cupuassu jelly without fruit pieces	Cupuassu jelly with fruit pieces
Chromium (µg/g)	0.70	0.40	0.40	0.10	0.10	0.10	0.10
Copper (µg/g)	2.40	1.70	1.20	1.20	0.90	5.60	1.30
Manganese (µg/g)	45.20	37.50	4.90	3.20	1.00	1.10	0.60
Nickel (µg/g)	0.50	0.30	0.30	0.10	0.10	0.10	0.00
Lead (µg/g)	0.01	0.01	0.01	0.07	0.06	0.07	0.08
Zinc (µg/g)	2.30	2.00	1.90	1.40	0.80	1.40	1.00

Conclusion:

The highest concentration of chromium found in the products was 0.70 µg/g, in acai pulp. In relation to cupuassu, the concentration of 0.1 µg/g was found in all products. To be considered as toxic, chromium has to be ingested in doses above 100 µg/g. In relation to copper, the highest concentrations found were 2.40 µg/g in acai pulp and 5.60 µg/g in cupuassu jam without fruit pieces. To be considered toxic in fruits and vegetables copper must be found above 1000 µg/100 g. In respect of manganese, the highest concentrations were 45.20 µg/g in acai pulp. In the cupuassu products, the highest value was 3.20 µg/g in pulp. The limit of manganese to avoid adverse effects is 150.00 µg per day. The highest values of nickel found in the analysis were 0.50 µg/g in acai pulp. In relation to cupuassu, the highest concentration of 0.1 µg/g was found in pulp, jam and jelly without fruit pieces. The recommended amount of nickel is between 5.00 and 15.00 µg per day. The highest value of lead found in this study was 0.08 mg/g in cupuassu jelly with fruit pieces. In relation to acai, the highest concentration of 0.01 µg/g was found was found in all products. The acceptable limits of lead in foods range from 0.05 to 2.00 µg/g. In relation to zinc concentrations, among the acai products, pulp had the highest concentration, 2.30 µg/g. Among the cupuassu products, the highest concentration was observed also in pulp, 1.40 µg/g. Only very high levels of zinc intake above 1,500 µg per day can cause nutritional imbalance in the human body. Processed products of acai and cupuassu fruits produced by RECA showed no significant amounts of toxic elements that could endanger consumer health. This research can support future studies concerning other products from Amazonian biodiversity.

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